BIODEGRADATION OF BIO-BASED PLASTICS AND ANAEROBIC DIGESTION OF CAVITATED MUNICIPAL SEWAGE SLUDGE

DISSERTATION

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Abstract

Two areas that represent major challenges and opportunities for petroleum-based substitutes include production of plastics and energy sources. Recently, significant progress has been made in developing plastics made from renewable biomass feedstocks. However, studies on the potential products of naturally occurring degradation processes and their interaction with the environment have not been performed. In terms of production of renewable energy sources from biomass, anaerobic digestion (AD) is an important component in modern wastewater treatment plants because it can transform part of the organic material into biogas. However, biogas production maximization and conversion of recalcitrant and depleted sludge remain major limitations. Two studies on the biodegradability of bio-based plastics under different conditions and one of anaerobic digestion of cavitated sewage sludge were conducted to address these gaps of knowledge. In the first study, the relative biodegradability of a wide range of commercially available plastic alternative materials were determined during composting, AD and soil incubation. In the second study, the relative biodegradability and structural changes of polyurethane foams (PUs) made from petroleum and bio-based polyols were compared under the three conditions aforementioned. In the third study, the ability of hydrodynamic cavitation to reduce the particle size of sewage sludge obtained from three different full-scale wastewater treatment facilities in Ohio, US were evaluated. Biomethanation studies were
also conducted to evaluate process stability and performance in mesophilic anaerobic digesters treating cavitated sludge. The first study demonstrated that although certain bio-based plastics and natural fibers were biodegraded to an appreciable extent in the three environments, only a polyhydroxyalkanoate-based resin biodegraded to significant extents during the time scale of composting and anaerobic digestion processes used for solid waste management. Moreover, no significant degradation was observed for polyethylene or polypropylene plastics or the same plastics amended with additives that are claimed to facilitate biodegradability. The second study showed that overall, PUs degraded very slowly or not at all in composting, AD and soil environments. However, PUs made from bio-based polyols did show evidence of limited biodegradation during soil incubation. Thermogravimetric Analysis (TGA), Evolved Gas Analysis Mass Spectrometry (EGA-MS) and Fourier Transform Infrared Spectroscopy (FT-IR) analysis conducted on the PUs before and after composting revealed that important structural changes occurred in the PUs containing at least 50% of bio-based derived polyols. Further analysis revealed that both urethane and ester-related segments were susceptible to microbial attack in samples containing at least 50% of bio-based polyols. Moreover, ester groups in the polyol side of the polymer were the preferred site of attack in PUs made from 100% bio-based polyols. The third study revealed that hydrodynamic cavitation produced substantial reductions in the particle size of all the feedstocks studied that had total solids contents ranging from 0.38% and 3.55%. In contrast, reductions in particle size were marginal when a feedstock containing 5.21% total solids was processed at mild cavitation process conditions. Laboratory-scale batch AD conducted with
feedstocks containing mostly secondary sludge showed that cavitation pretreatment increased total biogas production and volatile solids reduction by 66.8 and 34.2%, respectively. However, AD of samples consisting of mostly primary sludge did not result in significant improvements in biogas production.
Dedication

To my loving wife Giovana D. Mercali, my parents Eddie Antonio and Marielos, family and friends.
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Major Field: Food, Agricultural, and Biological Engineering
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Chapter 1 - Introduction

Concerns about the depletion, growing demand and prices, and environmental impacts of fossil fuels has resulted in investigations of substitutes produced from renewable resources that can meet the cost and performance requirements of the market (Babb, 2012). This represents a major challenge and opportunity for this billion dollar industry. One of the most appealing substitutes is biomass. Biomass is an abundant and renewable resource that can be converted into different chemicals, transportation fuels and other value-added materials (Klass, 1998).

Recently, significant efforts have been made to develop plastics that are made from renewable biomass feedstocks (Song et al., 2009). Whereas much of the attention has been given to different production methods and to properties that satisfy different applications, the biodegradability of the products and their interaction with the environment have been neglected (Babb, 2012; Narayan, 2011). Therefore, there is a research need to understand how and if these new materials degrade during common waste management methods such as composting and anaerobic digestion (AD).

Other research efforts are focused on finding substitutes for fossil fuels that can produce energy (Chum & Overend, 2001). AD converts organic residues into different forms of energy and is a main component of many modern wastewater treatment plants (Ponsá et al., 2008). One of the main challenges has been augmenting the conversion of recalcitrant substrates which limit the hydrolysis stage of the overall AD process
Even though improvements in solubilization and disintegration rates have been achieved using different methods of pretreatment, factors such as long retention times and energy consumption have limited its applicability in full-scale systems (Pilli et al., 2011). A simple and low cost alternative is hydrodynamic cavitation (Harrison & Pandit, 1992). However, research on this area is scarce. Hence, there is a need to understand the effects of cavitation on substrate characteristics and AD performance of different substrates.

To address these needs, two studies on biodegradability of bio-based plastics under different conditions and one of anaerobic digestion of cavitated sewage sludge were conducted. The first study determined the relative biodegradability of a wide range of commercially available plastic alternative materials during composting, AD and soil incubation. The second study determined the relative biodegradability of polyurethane foams (PUs) produced from petroleum and bio-based polyols during composting, AD and soil incubation. Structural changes in the PU foams were analyzed using Thermogravimetric Analysis (TG), Evolved Gas Analysis Mass Spectrometry (EGA-MS) and Fourier Transform Infrared Spectroscopy (FT-IR) before and after composting. The third study evaluated the ability of hydrodynamic cavitation to reduce the particle size of sewage sludge obtained from three different full-scale wastewater treatment facilities in Ohio, US. Biomethanization studies were then conducted to evaluate process stability and performance in mesophilic anaerobic digesters treating the cavitated sludge.
Chapter 2 - Biodegradability of Conventional and Bio-based Plastics and Natural Fiber Composites during Composting, Anaerobic Digestion and Long-term Soil Incubation

Abstract

Plastics are a major constituent of municipal solid waste and pose a growing environmental pollution problem. To reduce their environmental impacts various substitute materials have recently been introduced that improve the biodegradability of plastics. These include conventional plastics amended with additives that are meant to enhance their biodegradability, as well as bio-based plastics and natural fiber composites made from renewable feedstocks. In this study, the rate and extent of mineralization of a wide range of commercially available plastic alternative materials were determined during composting, anaerobic digestion and soil incubation. The biodegradability was assessed by measuring the amount of carbon mineralized from these materials during incubation in the three environments. During soil incubation for 660 days, substantial mineralization was observed for polyhydroxyalkanoate plastics, starch-based plastics and for compost based materials. However, only a polyhydroxyalkanoate-based plastic biodegraded at a rate similar to the positive control (cellulose). No significant degradation was observed for polyethylene or polypropylene plastics or the same plastics.
amended with commercial additives meant to confer biodegradability. During 50 days of anaerobic digestion, 20-25% of the bio-based materials but less than 2% of the additive containing plastics were converted to biogas (CH$_4$ + CO$_2$). After 115 days of composting, 0.6% of an additive amended polypropylene, 50% of a plastarch material and 12% of a soy wax permeated paper pulp was converted to carbon dioxide. Scanning electron microscopy showed substantial disintegration of polyhydroxyalkanoate-based plastic, some surface changes for other bio-based plastics and coconut coir materials but no evidence of degradation of polypropylene or polypropylene containing additives. Although certain bio-based plastics and natural fibers biodegrade to an appreciable extent in the three environments, only a polyhydroxyalkanoate-based resin biodegraded to significant extents during the time scale of composting and anaerobic digestion processes used for solid waste management.

**Introduction**

Plastics are synthetic and semi-synthetic polymeric compounds, derived primarily from fossil carbon sources such as crude oil and natural gas. Their mechanical properties and characteristics such as low-cost, durability and processability, have led to their widespread use for diverse applications. However most commonly used plastics are very resistant to biological degradation (Chum, 1991). This has led to major challenges for waste management operations that are moving toward more sustainable waste management practices including recycling, composting and anaerobic digestion.
It is estimated that of the 31 million tons of plastic waste generated annually in the U.S. only 8% is recycled (U.S. EPA, 2011). Therefore, a large percentage of plastic waste is currently landfilled, or released into the environment. Throughout the world, roadsides, parks, beaches, oceans and natural areas are inundated with plastic debris pollution (Hammer et al., 2012). Waste management systems are also affected by high volumes of plastics that are often commingled with organic wastes (food scraps, wet paper, yard trimmings, soil and liquids), making it difficult and impractical to recycle both organic fractions and/or the plastics mixed with them without expensive cleaning, separation and sanitizing procedures (Hopewell et al., 2009). This has resulted in global concerns and intensive efforts to develop plastic materials that not only have acceptable prices and similar performance but also are made from renewable feedstocks and/or undergo biodegradation in a reasonable amount of time without leaving toxic residues (Song et al., 2009).

Although biodegradable bio-based plastics are meant to improve the sustainable use of resources, many aspects of a complete life-cycle analysis must be conducted (Narayan, 2011) to insure that the solution is not worse than the problem. For example, many factors greatly impact the life-cycle carbon balance of plastics including the source of the feedstock used to make them, whether the material is recycled and the extent of biodegradation during disposal. For example, most plastics are derived largely from fossil sources such as natural gas or crude oil (Maier et al., 1998). However the monomers used to make them can also be made from renewable resources. In Brazil, ethylene, the building block of one of the most widely used plastics, polyethylene (Braskem, 2012) is made from sugar cane converted to ethanol. Although made from a biomass feedstock,
this type of polyethylene is still essentially not biodegradable. On the other hand, petroleum can also be used to make plastics that are biodegradable. The lactic acid used to make polylactic acid (PLA) can be produced both by fermentation and synthetically from petroleum (McKetta & Cunningham, 1976), and either type is biodegradable. On this basis, plastics can be classified into four types; plastic, bio-based plastic, biodegradable plastic and biodegradable bio-based plastic (Table 1).

<table>
<thead>
<tr>
<th>Class</th>
<th>Source</th>
<th>Biodegradable</th>
<th>Example</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Petroleum/Natural Gas.</td>
<td>No</td>
<td>Polyethylene, Polypropylene.</td>
<td>(Maier et al., 1998)</td>
</tr>
<tr>
<td>II</td>
<td>Petroleum/Natural Gas.</td>
<td>Yes</td>
<td>PLA&lt;sup&gt;a&lt;/sup&gt; from petroleum.</td>
<td>(McKetta &amp; Cunningham, 1976)</td>
</tr>
<tr>
<td>III</td>
<td>Biomass (Corn, sugar cane, etc).</td>
<td>No</td>
<td>Polyethylene derived from corn ethanol.</td>
<td>(Braskem, 2012)</td>
</tr>
<tr>
<td>IV</td>
<td>Biomass (Corn, sugar cane, etc).</td>
<td>Yes</td>
<td>PHA&lt;sup&gt;b&lt;/sup&gt;, PLA derived from starch.</td>
<td>(Vroman &amp; Tighzert, 2009)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Polylactic acid. <sup>b</sup>Polyhydroxyalkanoates-based resin.

Understanding the benefits and proper uses of these four classes of materials (Table 1) and the impact of their use on atmospheric carbon dioxide (CO₂) accumulation can be confusing and is not always straightforward. Plastics made from petroleum, such as polyethylene, have a well-defined life cycle. When landfilled, the carbon in the plastic will be sequestered and not contribute to global warming. Recycled polyethylene may contribute even less fossil CO₂ to the environment if less energy is used to recycle it than
is used to make it in the first place. In this case, conventional plastics may have much less impact on GHG emissions that those designed to biodegrade.

For reasons presented above, efforts have been made to develop plastics made from renewable biomass feedstocks (Song et al., 2009). These are called “bio-based plastics”. On balance this type of plastic offers a great potential to reduce greenhouse gases in the atmosphere by sequestering carbon. This is because atmospheric CO$_2$ is fixed into the carbohydrates used as their feed stock. If the plastic is eventually landfilled, this carbon will become locked for millennia within the landfill and on balance reduce atmospheric CO$_2$. However these plastics also pose pollution problems (Weiss et al., 2012).

Biodegradable bio-based plastics, are also made from biomass but are designed to be compostable and/or biodegradable. These types include PLA and polyhydroxyalkanoates-based resins (PHA) made from corn. This class of polymer is carbon neutral from the standpoint of the carbon in the plastic, but a substantial amount of fossil energy is used to produce the plastic and the biomass feedstocks.

The class with perhaps the greatest potential to contribute to greenhouse gas emissions is biodegradable plastics made from petroleum. This is because not only is fossil energy used to produce them in the first place, but fossil carbon is released when the material ultimately biodegrades. If this biodegradation occurs in a landfill, then it usually will generate methane (CH$_4$), which is a greenhouse gas with 21 times the warming potential of CO$_2$. Most landfills do a poor job of capturing this gas, even those with methane recovery systems (Bogner & Matthews, 2003). So landfilled biodegradable
plastics, eventually contribute with both methane and carbon dioxide to the atmosphere when they degrade.

Some novel polymers combine both biomass and fossil derived resins to decrease production prices, increase the bio-based content and improve material performance (Song et al., 2009) (e.g. a plastarch containing a blend of a starch-based polymer and conventional plastics such as polypropylene). The biogenic renewable carbon contained in these and other biomaterials can be determined from the radioactive C\textsubscript{14} signature of the product (Narayan, 2006). Yet these hybrid materials likely are neither recyclable nor completely biodegradable and therefore are likely worse than conventional plastics from a GHG emissions perspective.

Composting plays an important and growing role in sustainable organic waste management and recycling. However, plastics are one of the main contaminants in composts. Biodegradable plastics are meant to address this problem. Composting of these materials also reduces their environmental impact in that they will largely be converted to CO\textsubscript{2} and not to CH\textsubscript{4} as they would be in a landfill. Since this CO\textsubscript{2} was originally fixed from the atmosphere into renewable biomass, on balance it will not increase atmospheric CO\textsubscript{2}.

Biodegradation is the mineralization of materials as a result of the action of naturally-occurring microorganisms such as bacteria and fungi (ASTM, 2011). The biodegradation of plastics is limited by their molecules weight, chemical structure (Vroman & Tighzert, 2009), water solubility and the fact that most plastics are xenobiotic. That is, they were not present in the environment until very recently so that
the evolution of metabolic pathways necessary for their biodegradation, a process that takes millions of years, has yet to occur.

In contrast, the biodegradation of natural polymers, such as starch or cellulose by microorganisms occurs relatively rapidly. It begins with the excretion of extracellular enzymes that depolymerize these materials. Once the polymer is reduced to a size that is water soluble and able to be transported through the cell wall, microbial metabolic pathways can then mineralize it (Albertsson, 1993). Even though microorganisms drive the biodegradation process, other non-biotic chemical processes such as photo-oxidation and chemical degradation may also take place before or in parallel.

Biodegradable materials are used in diverse applications. Many different biodegradable plastics are used for food packaging and for waste containment. They have also been developed for medical applications, including medical devices and for drug delivery (Shalaby et al., 1995). Biodegradable plastics are used widely in agriculture, as mulching films and low tunnels (Briassoulis & Dejean, 2010; Briassoulis et al., 2010) as well as guide strings and plant nursery containers (Lopez & Camberato, 2011). The physical properties and performance of biodegradable plastics made from PLA and natural fibers were found to be similar to conventional plastics for greenhouse crop production (Evans et al., 2010). In addition, biodegradable potting containers have gained a high degree of acceptance among consumers (Hall et al., 2010).

Recently, various materials have begun to be marketed that claim to be biodegradable or compostable. Terms such as “degradable”, “oxo-biodegradable”, “biological”, “compostable” and “green” are often used to describe and promote different plastics. These materials include conventional plastics amended with additives meant to
enhance biodegradability as well as bio-based plastics and natural fiber composites. There has been little research on the extent to which these materials truly degrade and/or biodegrade over the time scale of waste management systems such as composting facilities and anaerobic digestion (AD) or in natural settings (Edwards, 2013).

The objective of this study was to compare the relative biodegradability of a range of novel plastics and natural fiber composites during composting, AD and in soil conditions. The hypothesis is that materials that are referred to as biodegradable, compostable (or similar terms), and plastics containing additives designed to enhance biodegradability, mineralize during the time scale of waste treatment processes and in reasonable amounts of time in the environment and at rates comparable to natural materials known to be biodegradable and or compostable (e.g. cellulose paper).

Materials and methods

Standardized laboratory-scale experiments were conducted to study the biodegradability of various materials during soil incubation, composting and AD conditions (ASTM, 2003a; ASTM, 2003b; ASTM, 2002). Equation 1 was used to calculate the extent of biodegradation (\(X\)) where \(C_s\) is the average carbon (\(\text{CO}_2\) and or \(\text{CH}_4\)) mineralized from each treatment, minus the average carbon evolved from blanks \((Cb)\), and dividing this by the total amount of sample carbon added to each treatment \((Ca)\). Reactors containing only the inoculum (AD), soil (soil tests) or compost (compost tests) were used as blanks.
\[ \chi = \frac{C_S - C_B}{C_A} \]  

Materials

Test specimens included plastics designed to be biodegradable, conventional plastics amended with additives that are meant to enhance biodegradability, bio-based plastics and natural fiber composites (Table 2 and Table 3). The positive and negative controls used for all experiments were cellulose paper (Fisher Scientific, PA, U.S.) and 100% conventional polypropylene (PP), respectively. Materials were tested both after grinding (a preliminary soil experiment only) and as 1x1 cm squares (thicknesses shown in Table 3).
Table 2. Material information for commercially available bio-based plastics, plastics amended with additives and natural fiber composites.

<table>
<thead>
<tr>
<th>Material</th>
<th>Material Description</th>
<th>Formation process&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP + 2% additive</td>
<td>Blend of polypropylene (PP) with 2% ECM MasterBatch Pellets™ additive (ECM BioFilms Inc., OH, U.S.).</td>
<td>1</td>
</tr>
<tr>
<td>PS + 2% additive</td>
<td>Blend of polystyrene (PS) with 2% ECM MasterBatch Pellets™ additive (ECM BioFilms Inc., OH, U.S.).</td>
<td>1</td>
</tr>
<tr>
<td>PETE + 1% additive</td>
<td>Blend of polyethylene terephthalate (PETE) with 1% EcoPure® additive (Bio-Tec Environmental LLC., NM, U.S.).</td>
<td>2</td>
</tr>
<tr>
<td>Plastarch</td>
<td>A blend of polypropylene with corn starch.</td>
<td>3</td>
</tr>
<tr>
<td>Co-polyester + corn-based plastic</td>
<td>Blend of an aliphatic aromatic co-polyester with a corn starch-derived polymer (Ecobra's™, BASF).</td>
<td>1</td>
</tr>
<tr>
<td>PHA</td>
<td>Made from polyhydroxyalkanoates-based resin (Metabolix, MA, U.S.).</td>
<td>1</td>
</tr>
<tr>
<td>Paper pulp + soy wax</td>
<td>Paper pulp pot permeated with soy wax.</td>
<td>4</td>
</tr>
<tr>
<td>Paper pulp</td>
<td>Recycled (74% minimum) paper pulp.</td>
<td>4</td>
</tr>
<tr>
<td>Paper pulp + asphalt</td>
<td>Blend of recycled (74% minimum) paper pulp + asphalt.</td>
<td>4</td>
</tr>
<tr>
<td>Coconut coir</td>
<td>Made from coconut husk.</td>
<td>7</td>
</tr>
<tr>
<td>Rice hull</td>
<td>Made from rice hull.</td>
<td>5</td>
</tr>
<tr>
<td>Composted cow manure</td>
<td>Made from composted cow manure.</td>
<td>6</td>
</tr>
<tr>
<td>Peat fiber</td>
<td>Made from Canadian sphagnum peat moss + wood pulp.</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup>1=Injection molding; 2=Blow molding; 3=Thermoforming; 4=Vacuum forming; 5=Compression forming; 6=Pressure forming; 7=Other.
Table 3. Chemical and physical properties of the test specimens.

<table>
<thead>
<tr>
<th>Material</th>
<th>Total solid (% ww)</th>
<th>Volatile solids (% dw)</th>
<th>Total carbon (% dw)</th>
<th>Total nitrogen (% dw)</th>
<th>Film Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>90.3±5</td>
<td>57.4±1.1</td>
<td>41.8±0.1</td>
<td>0.03±0.01</td>
<td>0.35±0.01</td>
</tr>
<tr>
<td>Negative</td>
<td>99.8±0.1</td>
<td>96.3±2</td>
<td>82.9±0.1</td>
<td>0.06±0.003</td>
<td>0.37±0.01</td>
</tr>
<tr>
<td>PP + 2% additive</td>
<td>99.8±0.1</td>
<td>97.7±0.1</td>
<td>82.9±0.3</td>
<td>0.04±0.01</td>
<td>0.37±0.03</td>
</tr>
<tr>
<td>PS + 2% additive</td>
<td>99.9±0.1</td>
<td>97.0±1.5</td>
<td>88.8±1</td>
<td>0.05±0.01</td>
<td>0.23±0.01</td>
</tr>
<tr>
<td>PETE + 1% additive</td>
<td>99.4±0.5</td>
<td>99.9±0.1</td>
<td>64.6±0.1</td>
<td>0.01±0.002</td>
<td>0.36±0.01</td>
</tr>
<tr>
<td>Plastarch</td>
<td>90.9±2.1</td>
<td>57.5±3</td>
<td>60.9±0.2</td>
<td>0.07±0.01</td>
<td>0.48±0.03</td>
</tr>
<tr>
<td>Co-polyester + corn-based plastic</td>
<td>95.2±0.1</td>
<td>99.8±0.1</td>
<td>51.9±0.3</td>
<td>0.10±0.01</td>
<td>0.72±0.02</td>
</tr>
<tr>
<td>Wheat starch-derived plastic</td>
<td>97.8±0.4</td>
<td>98.5±0.5</td>
<td>49.4±0.1</td>
<td>0.74±0.004</td>
<td>0.50±0.01</td>
</tr>
<tr>
<td>PHA</td>
<td>99.4±0.4</td>
<td>90.4±0.5</td>
<td>50.7±0.3</td>
<td>0.45±0.01</td>
<td>0.62±0.01</td>
</tr>
<tr>
<td>Paper pulp + soy wax</td>
<td>94.3±1</td>
<td>91.0±0.4</td>
<td>46.9±0.3</td>
<td>0.06±0.01</td>
<td>2.14±0.03</td>
</tr>
<tr>
<td>Paper pulp</td>
<td>92.0±0.1</td>
<td>92.0±0.1</td>
<td>42.1±0.1</td>
<td>0.10±0.01</td>
<td>2.74±0.01</td>
</tr>
<tr>
<td>Paper pulp + asphalt</td>
<td>93.4±0.5</td>
<td>90.6±0.3</td>
<td>46.9±0.03</td>
<td>0.22±0.02</td>
<td>2.61±0.1</td>
</tr>
<tr>
<td>Coconut coir</td>
<td>96.8±0.3</td>
<td>98.5±0.5</td>
<td>46.7±0.3</td>
<td>0.26±0.002</td>
<td>1.09±0.02</td>
</tr>
<tr>
<td>Rice hull</td>
<td>94.0±0.4</td>
<td>89.6±0.4</td>
<td>38.3±0.1</td>
<td>14.1±0.06</td>
<td>1.24±0.02</td>
</tr>
<tr>
<td>Composted cow manure</td>
<td>92.5±0.1</td>
<td>89.4±1.0</td>
<td>40.5±0.01</td>
<td>1.12±0.05</td>
<td>2.40±0.1</td>
</tr>
<tr>
<td>Peat fiber</td>
<td>92.1±0.3</td>
<td>97.8±0.5</td>
<td>45.4±0.3</td>
<td>0.49±0.07</td>
<td>1.74±0.05</td>
</tr>
</tbody>
</table>

*Values are means ± standard deviation of three determinations.

**Biodegradation in Soil Incubation**

The extent of long-term biodegradation of polymeric materials in contact with soil was determined based on ASTM D5988-03 (ASTM, 2003a). These included PP + 2% additive, polystyrene (PS) + 2% additive, polyethylene terephthalate (PETE) + 1%
additive, plastarch, a co-polyester + corn-based plastic, a wheat starch-derived plastic and PHA (Table 2 and Table 3). Six natural fiber composite materials were also tested: paper pulp, paper pulp + asphalt, coconut coir, rice hull, composted cow manure and peat fiber. All samples were incubated in triplicate for a period of 660 days.

The soil media used for the experiments was a mixture of 43% certified organic top soil, 43% no-till farm soil collected at coordinates: 40.778633, -81.930873 and 14% sand. Soil was sieved to less than 2 mm particle size and large plant materials, stones, and other inert materials were removed. The chemical properties of the soil mixture are shown in Table 4. The soil media was amended with ammonium phosphate (Fisher Scientific, PA, U.S.) to maintain a C:N ratio of 20:1 based on the carbon content of the test specimen.

The soil mixture (300 g dry) was placed in the bottom of a 2-liter (working volume) wide mouth jar (Ball® Corporation, item # 383178). Distilled water was added to bring the moisture content of the mixture to 60% of the moisture holding capacity. The test specimens (1 g of sample carbon) were then mixed thoroughly into the soil. A solution containing 20 ml of potassium hydroxide (KOH) 0.5N (Fisher Scientific, PA, U.S.) was placed in a cup suspended from the lid of each vessel to trap evolved CO2. All vessels were sealed and incubated at room temperature (20±2°C).
Table 4. Initial mean characteristics of the aerobic and anaerobic organic substrates.

<table>
<thead>
<tr>
<th>Organic substrate</th>
<th>Chemical and physical properties&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total solids (%) ww</td>
<td>Volatile solids (%) dw</td>
<td>Total carbon (%) dw</td>
<td>Total nitrogen (%) dw</td>
<td>pH</td>
</tr>
<tr>
<td>Compost&lt;sup&gt;b&lt;/sup&gt; inoculum</td>
<td>24.3±2.0</td>
<td>88.9±1.0</td>
<td>48.7±5.5</td>
<td>2.37±0.2</td>
<td>7.95±0.04</td>
</tr>
<tr>
<td>Soil mixture&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87.4±0.1</td>
<td>2.96±0.1</td>
<td>1.19±0.2</td>
<td>0.13±0.02</td>
<td>7.43±0.4</td>
</tr>
<tr>
<td>Anaerobic seed&lt;sup&gt;d&lt;/sup&gt; sludge MEN</td>
<td>8.92±0.5</td>
<td>59.5±2.0</td>
<td>36.8±1.0</td>
<td>7.21±0.2</td>
<td>8.30±0.01</td>
</tr>
<tr>
<td>Medina County&lt;sup&gt;e&lt;/sup&gt; OFMSW</td>
<td>47.2±7.2</td>
<td>60.3±1.2</td>
<td>89.6±1.3</td>
<td>0.92±0.2</td>
<td>7.50±0.4</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± standard deviation of three replicates. <sup>b</sup>Dairy manure and hardwood sawdust mature compost; <sup>c</sup>This is the value before adding water to reach 60% of the water holding capacity; <sup>d</sup>Methanogenically active municipal sewage sludge; <sup>e</sup>OFMSW = the organic fraction of municipal solid waste.

Carbon dioxide produced in each vessel reacted with the KOH in the cup to form potassium bicarbonate. The amount of CO₂ produced was determined by titrating the KOH solution with 0.25N hydrochloric acid (Fisher Scientific, PA, U.S.) to a phenolphthalein end-point. The experiment was designed so that the headspace volume was sufficient to prevent the oxygen concentration in the vessel from falling below 18%. The KOH traps were removed and titrated at time intervals that assured that their absorption capacity was not exceeded. The KOH traps were refilled at a rate dependent on the rate of CO₂ generation in each flask. At the time of removal of the traps, the vessel was flushed and allowed to sit open to allow fresh air to fill the headspace. In addition, distilled water was added to the soil to the original weight to maintain adequate moisture.

The effect of particle size on biodegradation rate was determined by comparing the biodegradability of 1 cm squares to ground samples. Samples were ground in liquid
nitrogen using a IKA® A11 basic Analytical mill (IKA® Works Inc., NC, U.S.) for 10 seconds. Test specimens included PP + 2% additive, co-polyester + corn-based plastic, wheat starch-derived plastic, paper pulp, paper pulp + asphalt, coconut coir and rice hull (Table 2 and Table 3). Samples were incubated in triplicate for 660 days.

**Biodegradation during Composting**

Three materials were tested under simulated composting conditions. These included PETE + 1% additive, plastarch and paper pulp + soy wax (Table 2 and Table 3). The experiments were conducted in triplicate for a period of 115 days.

The test conditions used were based on a protocol described in ASTM D5338-98 (2003) (ASTM, 2003b). This test is a measure of the degree and rate of carbon conversion to CO₂ under conditions that mimic a commercial scale industrial composting facility.

An 80 g sample of each test specimen was mixed with 350 g dry of mature compost inoculum (Table 4). The compost inoculum was obtained from a full-scale windrow composting facility featuring a concrete surface and controlled aeration system at OARDC. The compost contained a mixture of dairy manure and hardwood sawdust as described elsewhere (Michel *et al.*, 2004).

The compost was collected at various locations on the windrow and screened to less than 10 mm and large inert items were discarded. The screened compost was amended with ammonium phosphate (Fisher Scientific, PA, U.S.) to give a C:N ratio of
20:1 including the carbon content of the test specimen. The initial moisture content of the mixture was adjusted to 60% (wet-weight basis).

The compost and test specimens were incubated in 4-liter (working volume) vessels (length 30 cm and diameter 15 cm), made of PVC pipe placed in a 55°C incubator (BioCold Environmental Inc., MO, U.S.). Each vessel contained approximately 1100 g of material on a wet-weight basis. The reactors were aerated from below at 100±1 ml/min to maintain aerobic conditions. To avoid drying during the experiment, air was saturated by bubbling through bottles containing water at the incubator temperature. The air exiting the vessels was passed through flasks in a separate water bath set at 9°C to condense moisture from the off-gas. The off-gas was then analysed for percent CO$_2$ using an infrared gas analyzer (Vaisala model GMT 220, range 0 to 20%). CO$_2$ data was automatically recorded using a Campbell Scientific model 23XL data logger for each vessel every hour. Each vessel was also equipped with a K-type thermocouple to measure the temperatures of the composts mix near the center of the compost vessel, and was recorded automatically every 12 minutes. A more complete description of the laboratory-scale composting system (Appendix A) can be found elsewhere (Grewal et al., 2006).

**Biodegradation during Anaerobic Digestions**

The biodegradation of four materials was compared during high solids batch anaerobic digestion. These included PP + 2% additive, PETE + 1% additive, plastarch and a co-polyester + corn-based plastic (Table 2 and Table 3). The experiments were conducted in triplicate for a period of 50 days.
The anaerobic degradation of the polymeric materials was compared under high-solids AD conditions based on a protocol described in ASTM D5511-02 (ASTM, 2002) international standard. The test measured the conversion of samples to CO₂ and CH₄ during incubation under controlled anaerobic conditions. For this study test specimens were exposed to an active methanogenic inoculum derived from a full-scale anaerobic digester treating municipal sewage sludge. These conditions resemble those found in high-solids AD digestors and in biologically active landfills, but not in typical landfills where water is excluded and removed.

The AD assays were conducted in 2-liter (working volume) laboratory-scale batch reactors. Temperatures were maintained at a mesophilic (37±1°C) range by means of incubators. Test specimens (25 g of sample carbon) were mixed with 750 g wet of methanogenically active sludge obtained in October of 2010 from a full-scale (3000 m³) anaerobic digester located at the City of Akron wastewater treatment plant and operated by KB Compost Services, Akron, Ohio (Gómez et al., 2011). This was mixed with 187.5 g wet of the organic fraction of municipal solid waste (OFMSW) of the Medina County, Ohio Solid Waste District to achieve the desired solids content for the test and to provide supplemental nutrients for the anaerobic microbial consortia. The chemical properties of the seed sludge and OFMSW substrate are shown in Table 4. Ammonium phosphate (Fisher Scientific, PA, U.S.) was added to the mixture to adjust the C:N ratio to a value of 20:1 considering the carbon content of the test specimen.

The volumetric production and CO₂ and CH₄ content of the biogas produced in the AD experiments was analysed by volume displacement and gas chromatography as
described by Gómez et al. (2011), respectively. This information was used to calculate the moles of carbon emitted from each reactor.

**Analytical Methods**

Solids content in soil, organic substrates and test specimens was determined by drying samples to a constant weight at 80°C. The volatile solids content was determined using an ashing oven set at 500°C for four hours. pH was determined using a pH electrode (TMECC 04.11-A 1:5 slurry method, mass basis). Carbon (TMECC 04.01-A combustion with CO₂ detection) and nitrogen content (TMECC 04.02-D oxidation, Dumas method) were determined by the Service Testing and Research laboratory at the OARDC.

Selected test specimens were also analyzed before and after soil incubation using scanning electron microscopy (SEM) (Hitachi S-3500N, Hitachi High Technologies America, Inc., CA, U.S.). Samples were coated with platinum to a thickness of 0.2kÅ using a Hummer® 6.2 sputtering system (Anatech USA, CA, U.S.). A 15Kv electron beam was applied.

**Statistical Analysis**

Three independent replicates were used for each treatment. Analysis of variance (ANOVA) was calculated for the average final cumulative percent of carbon loss for each of the studies. Comparisons for all pairs of final cumulative biodegradation means were performed using Tukey-Kramer HSD analysis. All conclusions were based on a
significant difference level of $\alpha=0.05$. The statistical analyses were performed using JMP statistical program version 9 (SAS Institute Inc., SAS Campus Drive, NC, U.S.).

Results and Discussion

*Biodegradation during Soil Incubation*

The importance of understanding the biodegradability of plastics in soil has increased since these are released inadvertently into the environment where they may persist. Plastics comprise a relatively large fraction of the ubiquitous pollution found worldwide in both land and ocean environments (Barnes et al., 2009). In addition, intensive and semi-intensive agriculture utilizes large quantities of these materials annually in the form of mulches, as plantable pots, nursery containers (Kyrikou & Briassoulis, 2007). This has resulted in the recent development of biodegradable agricultural plastics for these applications (Bastioli, 2001; Riggi et al., 2011). One example of this is biodegradable plant nursery pots. Some containers are designed to be plantable pots (e.g. rice hull and coconut coir) allowing them to degrade in the soil after planting, or to be composted at plant nurseries rather than being landfilled.

An initial experiment was conducted to assess the effect of particle size on biodegradation during soil incubation. Seven materials were tested and the amount of carbon converted to CO$_2$ was compared using student’s t method for particle size effect. Student’s t method revealed that out of the seven materials studied in this experiment, only one, a co-polyester + corn-based plastic, showed a significant effect of particle size on biodegradability. A significantly greater extent of biodegradation was observed for co-
polyester + corn-based plastic in 1x1 cm square film form (55.1±2.1%) after 660 days as compared to a ground sample of the same material (39.71±2.4%). For the rest of the materials, there was not a significant effect of particle size on biodegradation. Results from this study suggested that for most of the materials studied, biodegradability in soil was not greatly affected by particle size under the experimental conditions used in the study.

A second soil experiment was conducted to evaluate the relative biodegradability of thirteen different test specimens in 1x1 cm square film form. These included bio-based plastics, plastics amended with additives that are meant to enhance biodegradability and natural fiber composites. The experiment was conducted for a period of 660 days. The initial moisture content of the mixes was 16.6±2.1% and the final mean soil moisture content on a wet-weight basis across all treatments was 14.3±3.3% (wet-weight basis) which is 84.9±2.4% of the 60% moisture holding capacity of the soil mixture. The positive control (cellulose paper) exhibited 74.2±4.5% conversion during the period of incubation.

For some bio-based plastics and the positive controls (cellulose paper), the initial rate of mineralization was rapid (Figure 1). Most of the mineralization took place during the first 300 days of incubation (Figure 1). The most rapid initial rate of conversion was observed for co-polyester + corn-based plastic with almost 34.6±2.4% mineralized during the first 55 days of the experiment. The extent of PHA biodegradation was initially lower, but its extent surpassed that of co-polyester + corn-based plastic after approximately 280 days reaching a value of 48.5±4.6%. For the wheat starch-derived plastic and plastarch
conversion rates were 14.2±0.8 and 24.6±1.4% after 110 and 280 days of experiment, respectively.

Figure 1. Cumulative carbon loss (CO$_2$-C) as percentage of initial carbon (± cumulative standard error) for bio-based plastics and for conventional plastics amended with additives during 660 days of soil incubation. For some data points standard error bars are smaller than markers.

Final (660 days) cumulative biodegradation values during soil incubation for the positive control, PHA and co-polyester + corn-based plastic were 74.2±4.5, 69.2±6.4 and 55.1±6.1%, respectively. For the wheat starch-derived plastic and plastarch the final conversion reached 19.7±1.1 and 31.3±1.7%, respectively.

SEM images of PHA and co-polyester + corn-based plastic before and after mineralization showed substantial changes in the surface of the PHA material (Figure 2A and Figure 2B) and some degradation of the co-polyester + corn-based plastic (Figure 2C and Figure 2D).
For conventional plastics and the same plastics amended with additives that were supposed to enhance biodegradability, almost no biodegradation was observed after nearly two years of incubation in soil (Figure 1). The highest observed conversion during soil incubation was 1.0±0.1% (PP + 2% additive). For all other plastics amended with additives, the final cumulative biodegradation ranged between 0.9 and 1%. These values were less than that measured for the negative control (PP) which reached a final cumulative conversion of 1.3±0.7%. Although they were not significantly different. SEM images did not reveal qualitative changes in the appearance of PP or PP + 2% additive after the 2 year incubation period (Figure 2E and Figure 2F).
Figure 2. Scanning electron microscopy for selected materials during 2 years of soil incubation before and after biodegradation. From top to bottom: PHA (a: before, b: after), co-polyester + corn-based plastic (c: before, d: after), polypropylene + 2% additive (e: before, f: after) and coconut coir (g: before, h: after).
The mineralization in soil of the natural fiber composite materials was most rapid during the first 65 days of the experiment (Figure 3). This was followed by a period of slow mineralization until the termination of the experiment (Figure 3). After 660 days, the mineralization percent of the composted cow manure, paper pulp and paper pup + asphalt were 35.5±2.3, 31.3±3.6, 29.4±2.1%, respectively. Lower final conversion values were observed for rice hull, peat fiber and coconut coir with values of 21.1±2.6, 18.3±0.7 and 14.4±2.5%, respectively. SEM images of coconut coir revealed some surface changes indicative of biodegradation (Figure 2G and Figure 2H).

![Figure 3. Cumulative carbon loss (CO₂-C) as percentage of initial carbon (± cumulative standard error) for natural fiber composites during 660 days of soil incubation. For some data points standard error bars are smaller than markers.](image)

Approximately 74.2% of cellulose added to soil was converted to CO₂ after 660 days. This is similar to the conversion of cellulose of 80% reported in a 800 day soil
incubation conducted to evaluate how carbon substrates affect microbial biomass yield in soil biodegradation tests (Chiellini et al., 2007).

The highest biodegradability observed during soil incubation was reported for PHA (70%); a polyhydroxyalkanoate-based plastic. This was similar in magnitude to the extent of mineralization of the cellulose positive control (cellulose paper). Bacterial polyhydroxyalkanoates are intracellular aliphatic polyesters of various chain lengths (Kaplan, 1998). Several studies have been conducted to study the biodegradability of aliphatic polyesters under different conditions (Müller et al., 2001; Müller et al., 1998; Tokiwa & Calabia, 2007; Tokiwa et al., 2009). Mineralization of these polymers is mainly achieved by cleavage of the ester bonds which occurs due to both enzymatic and chemical hydrolysis (Tokiwa et al., 1990).

Statistical analyses revealed that significant differences in the extent of biodegradation ($F_{15,32}=822.2$, $P<0.0001$) existed between group means. Tukey-Kramer HSD analysis revealed that among bio-based plastics, the difference between PHA and the positive control (cellulose paper) was not significant. Analyses also revealed that differences were not significant between plastics amended with additives that are meant to enhance biodegradability and the negative control (PP). For natural fiber composites all test specimens differed significantly from both the positive and negative controls (Figure 3).

The results of this study indicate that plastics containing additives do not biodegrade any faster than non-additive containing plastics in soil. Manufacturers of these additives claim that if at least 1-5% (by weight) of their additive is added to plastics
products, these will fully biodegrade when disposed of in microbe-rich environments. These claims are not supported by the findings of this study.

The greatest extent of biodegradation among the fiber composite materials tested was the composted cow manure (35%). This was unexpected since low carbon conversion rates were anticipated for the composted cow manure since it had previously been biologically degraded. After undergoing a composting cycle, much of the carbon contained in the cow manure was expected to be stable and humified (Barrington et al., 2002; Michel et al., 2004). However, much less extents of degradation were observed for uncomposted composites produced from rice hulls, from peat fiber pot and coconut coir. For these materials, the extent of degradation in soil ranged from 14 and 21% (Figure 3). These materials have been used as natural composites due to their low price and structural strength (Mohanty et al., 2000; Saheb & Jog, 1999). Approximately 46% of coconut coir is lignin (Khedari et al., 2004) as is 21-40% of rice hulls (Pillaiyar, 1988) which may have limited their biodegradation.

**Biodegradation during Composting**

Three different materials were evaluated for their relative rate of degradation during composting. The materials were composted at 55°C under aerobic conditions for a period of 115 days. The tested materials included plastarch, paper pulp + soy wax and PETE + 1% additive (Table 2 and Table 3).

The initial moisture content was adjusted to 60% and the final mean compost moisture content across all treatments was 64.2 ±3.3% (wet-weight basis).
Mineralization under composting conditions occurred at a rapid initial rate for both the positive control and the plastarch material during the first 80 days (Figure 4). Overall, the positive control (cellulose paper) exhibited 78.4±3.5% conversion during composting.

For paper + soy wax, a majority of the mineralization took place during the first 15 days. For PETE + 1% additive no significant conversion was observed over the entire period of study (Figure 4). The final cumulative biodegradation during composting for plastarch, paper + soy wax and PETE + 1% additive was 51.3±4.9, 12.4±2.7 and 0.6±3.7%, respectively. The ANOVA indicated that statistically significant differences in the extent of biodegradation (F_{4,7}=496.6, P<0.0001) existed between group means. Tukey-Kramer HSD analysis revealed that all test specimens differed from the positive control. However, PETE + 1% additive did not differ significantly from the negative control.

None of the tested materials mineralized at rates comparable to the positive control material. The highest cumulative biodegradation during composting was observed for the plastarch containing material (51.3%). Starch is made of repeating glucose units linked by glucosidic bonds that are susceptible to enzymatic attack. Uses and applications of starch in its native form or blended with other materials have been discussed (Albertsson & Karlsson, 1995; Griffin Gerald J, 1974). Biodegradation of the starch containing portion of the material has been reported (Gould et al., 1990; Shah et al., 2008). However the reason that the plastarch degraded more slowly than cellulose is not known.
Figure 4. Cumulative carbon loss (CO$_2$-C) as percentage of initial carbon (± cumulative standard error) for bio-based plastics, conventional plastics amended with additives and natural fiber composites during 115 days of composting. For some data points standard error bars are smaller than markers.

After 20 days, only 12% of the paper pulp composite was converted to CO$_2$ during composting. The low level of cumulative degradation could be related to inhibitory properties of the soy derived wax on the microbial consortia or limiting water accessibility. For plastics containing additives, no degradation was observed. Additives did not improve the biodegradability of PETE during composting.

**Biodegradation during Anaerobic Digestion**

Understanding the biodegradation of different materials in anaerobic conditions such as in industrial sewage sludge AD systems, landfills and anoxic environments is important since under these conditions, microorganisms mineralize organic substrates to both CO$_2$ and methane. Methane itself can be used as a fuel source but if not captured it
has a global warming potential 21 times stronger than CO$_2$. Since in the U.S. only 30% of the landfills capture methane and among those that do capture, only a small percentage of the methane produced is recovered, then biodegradable plastics in landfills have a greater potential than composted biodegradable plastics to contribute to global warming.

The biodegradability of polymeric materials exposed to an active methanogenic inoculum was studied under controlled laboratory conditions that resemble those found during active AD for a period of 50 days. They likely differ somewhat from the conditions within a landfill where moisture is usually removed and a greater diversity of materials is present. Yet the extent of biodegradation is likely similar to what would ultimately occur over many years in a landfill environment.

Materials tested included plastarch, co-polyester + corn-based plastics, PP + 2% additive and PETE + 1% additive (Table 2 and Table 3). The mean methane content in the biogas across treatments during the entire period of study was 54.1±6.1%.

During the AD incubation, the positive control (cellulose paper) exhibited 74.1±4.8% conversion. For plastarch, the carbon conversion rate to biogas was similar to the positive control (cellulose paper) for the first 7 days (Figure 5). However, after this period, the rate of conversion slowed as compared to the positive control through day 28. In contrast, no significant mineralization was observed for the plastics containing additive samples over the entire period of the study.
The final cumulative carbon conversion during AD for plastarch and co-polyester + corn-based plastic were 26.4±3.5 and 20.2±4.4%, respectively. The final conversion values for PP + 2% additive and PETE + 1% additive were 3.1±3.7 and 2.2±1.6%, respectively. The ANOVA indicated that statistically significant differences in the extent of biodegradation (F_{5,12}=50.7, P<0.0001) existed between group means. The Tukey-Kramer HSD analysis revealed that the bio-based plastics were significantly different than the positive control but not different from each other. There was no significant difference in the carbon conversion of the negative control (PP) and the plastic containing the additive.

The biodegradability of different bio-based materials including cellulose and starch (Anderson, 2002; Rivard et al., 1992) has been investigated previously under...
anaerobic conditions (Abou-Zeid et al., 2004; Federle et al., 2002). Yagi et al. (2009) studied the biodegradability of cellulose powder under mesophilic (35°C) and thermophilic (55°C) AD conditions. Cellulose powder reached a cumulative conversion of 80% under both temperature conditions. Other authors have also studied the anaerobic mineralization of aliphatic polyesters. Abou-Zeid et al. (2001) conducted a study to determine the biodegradability of the natural polyesters poly(b-hydroxybutyrate) (PHB), poly(b-hydroxybutyrate-co-11.6%-b-hydroxyvalerate) (PHBV) and the synthetic polyester poly(o-caprolactone) (PCL) using different anaerobic sludges and individual strains. Biodegradability of the powdered materials was measured as the percent of weight loss. They found that almost all the PHB was converted in 9 days, but only 60 and 30% weight loss was observed for the PHBV and PCL, respectively. Similar results were reported by Shin et al. (1997) in which nearly complete conversion was observed for the natural bacterial polyester but no biodegradability for synthetic analogs were observed under simulated landfill conditions.

The results of this study indicate that materials have different rates of mineralization under different end of life scenarios. For example, the positive control reached 70% conversion in 25 days during AD while 75 and 400 days were needed to reach the same extent of conversion under composting and soil incubation conditions, respectively. The plastarch material degraded faster under composting conditions reaching 50% conversion in 85 days than under AD and soil incubation conditions where only 26 and 30% was converted after 50 and 660 days, respectively. For co-polyester + corn-based plastic 20% of the material was converted during 20 days of soil incubation while 50 days were needed to reach the same value during AD. Ultimately, co-polyester
+ corn-based plastic reached 55% conversion after 660 days of soil incubation. Conventional plastics and those containing additives did not degrade at all under any of the three conditions.

Biodegradable plastics are potential alternatives to petroleum-based materials that can be incorporated into organic recycling schemes based on anaerobic digestion or composting. They also could potentially reduce the pollution associated with conventional plastics and therefore lead to the development of products that are more environmentally friendly. Ideally, biodegradable materials must be useful for a predetermined service life and then biodegrade in a short period of time, leaving no visible fragments and no toxic residues when composted or anaerobically digested. Disposal of these materials in landfills as opposed to anaerobic digestions is not recommended since under anaerobic conditions they biodegrade to form methane and most landfills capture only a small fraction of the methane created (Levis & Barlaz, 2011).

Conclusion

In this study, the relative biodegradability of a range of polymeric materials and natural fiber composites used for various commercial applications was investigated under composting, soil incubation and anaerobic digestion conditions. The validity of the tests was confirmed in that positive controls (cellulose paper) biodegraded by more than 70% in all three systems in a reproducible manner.

While some of the bio-based plastics and natural fibers biodegraded to an appreciable extent, plastics containing additives that supposedly confer biodegradability
to polymers such as polyethylene and polypropylene did not improve the biodegradability of these recalcitrant polymers. SEM analysis confirmed that substantial biodegradation of polyhydroxyalkanoate-based plastics occurred and that some surface changes occurred in co-polyester + corn-based plastic and coconut coir materials. However, SEM confirmed that no degradation of polypropylene and polyethylene occurred, even after amendment with additives meant to confer biodegradability.

The relative biodegradability of the materials during long-term soil incubation was PHA > co-polyester + corn-based plastic > composted cow manure > plastarch > paper pulps > natural fibers > conventional plastics containing additives to enhance biodegradability = conventional plastics. For anaerobic digestion and composting the relative biodegradability was plastarch > co-polyester + corn-based plastic > conventional plastics with additives and plastarch > paper pulp + soy wax > conventional plastic with additives, respectively.

Over the timescale of organic recycling processes (composting and anaerobic digestion) most of the bioplastics biodegraded to only a limited extent. Furthermore, under anaerobic incubation, some of the bio-based plastics biodegraded to generate methane, a potent greenhouse gas that unless captured may negate the perceived environmental benefits of using these materials. Biodegradable plastics made from petroleum (Class II), may have more adverse environmental impacts than conventional plastics (Class I) if their ultimate fate is landfilling and anaerobic conversion to methane.
Chapter 3 - Biodegradability of Crude Glycerol-based Polyurethane Foams during Composting, Anaerobic Digestion and Soil Incubation

Abstract

In this study, the relative biodegradability of polyurethane foams (PUs) produced from bio and petroleum-based polyols during composting, anaerobic digestion, and soil conditions was compared. Structural changes in the PUs before and after composting were analyzed using Thermogravimetric Analysis (TGA), Evolved Gas Analysis Mass Spectrometry (EGA-MS) and Fourier Transform Infrared Spectroscopy (FT-IR). Mineralization studies showed that only the polyurethane foams from 100% bio-based derived polyols achieved biodegradation rates different to those observed for the petroleum-based analogs during 320 days of soil incubation. No significant differences in cumulative carbon losses between foams from blend polyols containing 50% bio-based polyols and the 100% petroleum-based counterparts were observed in any of the mineralization conditions. SEM analysis showed that some surface deterioration occurred in the PUs samples made from bio-based and blend polyols. Although some differences were observed in the TGA curves of the PUs made from petroleum-based polyols analyzed before and after composting, the most prominent differences in thermal decomposition behaviors occurred in both PUs made from bio-based and blend polyols in the thermal regions of the urethane and ester segments. In terms of EGA-MS analyses,
the major degradation of PUs made from bio-based and blend polyols was attributed to the decomposition of FAMEs and fatty acid chains in the polyol side of the polymer. FT-IR analysis showed that little degradation during composting in the petroleum-based PU foam occurred in both urethane and ester segments of the polymer. Studies conducted in the foams produced from 100% bio-based polyols revealed that the ester segments (C=O and C-O) of the material were the preferred sites of microbial attack and mainly contributed to their degradation. The PUs made from blend polyols showed some structural changes in the urethane linkages (N-H and C=O) and degradation was more noticeable in the ester segments (C=O and C-O) of the polymer similar to the 100% bio-based polyols.

**Introduction**

It is estimated that the total polyurethanes industry in the U.S. contributes with approximately $59.9 billion to the national economy output and the three major sectors of consumption include building and construction, transportation, and furniture and bedding (ACC, 2012). Polyurethanes foams (PUs), such as rigid and flexible foams, are one type of widely used polyurethanes products including insulation panels and seating cushions, adhesives, coatings, sealants, etc.

PUs are generally produced by the reaction of two major feedstocks, i.e., polyols and isocyanates. Currently, these feedstocks are heavily petroleum dependent (Desroches *et al.*, 2012). Global concerns about availability and increasing prices of petrochemical-derived products have led to investigations on substitutes that are not only produced from renewable sources, but also can meet the cost and performance requirements of the
market (Babb, 2012). This represents a major challenge and opportunity for renewable-based chemicals to supply this billion dollar industry.

Due to the limited choice of isocyanates, a majority of the research on renewable substitutes used for the production of PUs has focused on the polyol component. Polyols are alcohols containing two or more hydroxyl functional groups. Currently, most bio-based polyols (biopolyols) are produced from lignocellulosic biomass or vegetable oils (Li, 2011). Lignocellulosic feedstocks such as cornstalks, wheat straw and distillers’ grain have been studied for bio-based polyols (Liang et al., 2006; Xu J, 2012). Several vegetable oils including castor oil, soybean oil, and palm oil have been studied for production of bio-based polyols (Campanella et al., 2009; Cangemi et al., 2006). Recently, an increasing interest has been focused on the value-added uses of crude glycerol, a potential renewable substitute for petroleum-based feedstocks. Several studies have reported the production of crude glycerol-based biopolyols with suitable properties for PUs applications (Hu et al., 2012; Li & Zhou, 2011; Luo et al., 2013).

While significant achievements in production of PUs from bio-based polyols have been made, there are still numerous uncertainties about the interaction of these polymers with the environment. Understanding the biodegradability of PUs is particularly important as substantial amounts end up in waste management facilities every year and in natural environments (Hammer et al., 2012). From an engineering standpoint, biodegradability is desirable for single-use and short lifespan applications such as packaging materials while undesirable for long lifespan applications such as automotive and construction (Urgun-Demirtas et al., 2007). From an environmental standpoint, the potential products of biodegradability and their interaction with the environment need to
be accounted in addition to many other aspects of the materials life cycle (Narayan, 2011).

Biodegradation is the mineralization of materials as a result of the action of naturally-occurring microorganisms such as bacteria and fungi (ASTM, 2011). Degradation of PUs is limited by many factors including chemical properties of the polymer such as chemical structure, cross-linking density, the crystallinity, and the fact that most plastics are xenobiotic (Howard, 2002). Microbially-induced deterioration of the urethane bond in PUs has been reported to be influenced by the polyol segment type, i.e. polyether or polyester (Nakajima-Kambe et al., 1999). PUs made from polyether polyols have been found to be relatively resistant to microbial degradation, whereas the polyester analogs are reported to be more vulnerable to biodegradation processes (Darby & Kaplan, 1968). Differences in biodegradation rates have been attributed to the degradation mechanism in which endo and exo-type enzymatic depolymerization pathways are used for ester and ether PUs, respectively (Nakajima-Kambe et al., 1999). In polyesters, microbial degradation has been postulated to occur mainly due to hydrolysis of the ester bonds by membrane-bound and extra-cellular polyurethanases (Zheng et al., 2005).

Research has been conducted to evaluate the biodegradability of PUs made from vegetable oil-based and liquefied biomass-based polyols. Wang et al. (2008) studied the biodegradability by means of weight loss during soil incubation of PUs made from castor oil-derived polyols and reported reductions ranging between 10 to 40% in a four month period. In addition, microbial deterioration of PUs made from castor oil has been reported to occur in the ester bonds of the polymer (Cangemi et al., 2006). Shogren et al. (2004)
reported that degradation of PUs made from different vegetable oil polyols with hydrolysable bonds such as esters also biodegraded to an appreciable extent. Studies conducted by Zhang et al. (2012) reported 16% mass loss in a 12 month period during soil burial experiments and observed hydrolysis of the ester and urethane bonds due to microbial activity in PUs made from liquefied wood polyols. Weight losses ranging between 6 and 14% after 6 months of soil incubation were reported for PUs produced from liquefied waste paper (Lee et al., 2002) and wheat straw (Chen & Lu, 2009) and it was attributed to the biomass portion of the polymer.

Studies on the biodegradation of PUs produced from crude glycerol-based polyols have not been reported. More research needs to be conducted to understand the extent to which PUs made from crude glycerol polyols will biodegrade in different waste management scenarios such as composting and anaerobic digestion (AD) or in natural settings. The objective of this study was to compare the relative biodegradability of PUs produced from petroleum and bio-based polyols under composting, AD, and soil conditions. The hypothesis of this research is that PUs made from polyols with higher bio-based content are chemically different and will have greater biodegradation than their petroleum-based analogs.

**Materials and methods**

**Materials**

Petroleum-based (Stepanpol®) and bio-based (crude glycerol-derived) polyester polyols were provided by Bio100 Technologies, LLC. (Mansfield, OH). Chemical
properties of the polyols are shown in Table 5. Three types of PUs were prepared from petroleum, bio-based, and their blend polyols (50/50, w/w) following a commonly used formula (Tu et al., 2007) and ASTM D7487-08 (Table 6). The potential reactions in the production of PU foams from petroleum and bio-based derived polyols are shown in Figure 6.

Table 5. Properties of the petroleum and bio-based polyols used to produce the polyurethane foam samples.

<table>
<thead>
<tr>
<th>Polyols</th>
<th>Properties</th>
<th>Major components</th>
<th>Acid number(^b) (mg KOH/g(_{sample}))</th>
<th>Hydroxyl number(^b) (mg KOH/g(_{sample}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum-based</td>
<td></td>
<td></td>
<td>2.5±1</td>
<td>315±4</td>
</tr>
<tr>
<td>Bio-based</td>
<td></td>
<td></td>
<td>5±1</td>
<td>524±7</td>
</tr>
</tbody>
</table>

\(^a\)Oleic acid is used as a representative fatty acid in crude glycerol. \(^b\)The acid and hydroxyl numbers were determined in accordance with ASTM D4662-08 and ASTM D4274-05D, respectively. Values are means ± standard deviation of three determinations.
Table 6. Description of the formulas used to produce the experimental polyurethane foams.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Polyurethane foam production formula&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stepan polyol&lt;sup&gt;b&lt;/sup&gt; Bio-based polyol Isocyanate&lt;sup&gt;b&lt;/sup&gt; Polycat 5 Polycat 8 DABCO DC5357 Water</td>
</tr>
<tr>
<td>Petroleum-based PU</td>
<td>100 0 110 1.26 0.84 2.5 3</td>
</tr>
<tr>
<td>Bio-based PU</td>
<td>0 100 110 1.26 0.84 2.5 3</td>
</tr>
<tr>
<td>Blend PU</td>
<td>50 50 110 1.26 0.84 2.5 3</td>
</tr>
</tbody>
</table>

<sup>a</sup>parts by weight.  
<sup>b</sup>equivalent weight of polymeric MDI for isocyanate index.

PUs were cut in 10x10 mm squares with an average thickness of 2.1±0.4 mm for composting, AD, and soil incubation tests. Chemical and physical properties of the PUs are shown in Table 7. Cellulose paper (Fisher Scientific, PA, U.S.) was used as the positive control for all experiments.
Table 7. Initial characteristics of the positive control and polyurethane foam samples.

<table>
<thead>
<tr>
<th>Material</th>
<th>Chemical and physical properties&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total solids (%ww)</td>
</tr>
<tr>
<td></td>
<td>Volatile solids (%dw)</td>
</tr>
<tr>
<td></td>
<td>Total carbon (%dw)</td>
</tr>
<tr>
<td></td>
<td>Total nitrogen (%dw)</td>
</tr>
<tr>
<td>Positive control</td>
<td>90.3±5.0</td>
</tr>
<tr>
<td>(cellulose paper)</td>
<td>57.4±1.1</td>
</tr>
<tr>
<td></td>
<td>41.8±0.1</td>
</tr>
<tr>
<td></td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Petroleum-based</td>
<td>98.0±0.6</td>
</tr>
<tr>
<td></td>
<td>99.1±1.0</td>
</tr>
<tr>
<td></td>
<td>65.8±0.2</td>
</tr>
<tr>
<td></td>
<td>8.02±0.005</td>
</tr>
<tr>
<td>Bio-based</td>
<td>95.0±1.0</td>
</tr>
<tr>
<td></td>
<td>97.9±1.0</td>
</tr>
<tr>
<td></td>
<td>72.3±0.4</td>
</tr>
<tr>
<td></td>
<td>8.07±0.02</td>
</tr>
<tr>
<td>Blend</td>
<td>97.9±1.1</td>
</tr>
<tr>
<td></td>
<td>98.8±1.6</td>
</tr>
<tr>
<td></td>
<td>67.6±0.3</td>
</tr>
<tr>
<td></td>
<td>7.15±0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± standard deviation of three determinations.
Figure 6. Potential reactions between isocyanates and (a) bio-based polyols (oleic acid was used as a representative fatty acid in crude glycerol) and (b) petroleum-based polyols for the production of PU foams.
Biodegradation of Polyurethane Foams

Experiments to determine the biodegradability of PUs in contact with soil were conducted using an assay based on ASTM D5988-03. A more complete description of the methodology to study the mineralization of polymeric materials in soil can be found elsewhere (Gómez & Michel, 2013). The soil media used for the experiments was a mixture of 43% certified organic top soil, 43% no-till farm soil collected at coordinates: 40.778633, -81.930873 and 14% sand. The chemical properties of the soil mixture are shown in Table 8. The soil media was amended with ammonium phosphate (Fisher Scientific, PA, U.S.) to maintain a C:N ratio of 20:1 including the carbon content of the PU foam.

Table 8. Initial mean characteristics of the aerobic and anaerobic organic substrates.

<table>
<thead>
<tr>
<th>Organic substrate</th>
<th>Total solids (% ww)</th>
<th>Volatile solids (% dw)</th>
<th>Total carbon (% dw)</th>
<th>Total nitrogen (% dw)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost inoculum</td>
<td>43.7±0.8</td>
<td>71.5±12</td>
<td>38.9±3.5</td>
<td>2.98±0.4</td>
<td>8.19±0.02</td>
</tr>
<tr>
<td>Soil mixture</td>
<td>90.5±0.1</td>
<td>2.8±0.03</td>
<td>0.87±0.4</td>
<td>0.12±0.03</td>
<td>6.63±0.4</td>
</tr>
<tr>
<td>Anaerobic inoculum</td>
<td>9.02±0.04</td>
<td>62.0±0.3</td>
<td>38.8±1.6</td>
<td>6.30±0.2</td>
<td>8.06±0.07</td>
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<tr>
<td>Medina County OFMSW</td>
<td>47.2±7.2</td>
<td>60.3±1.2</td>
<td>89.6±1.3</td>
<td>0.92±0.2</td>
<td>7.50±0.4</td>
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<tr>
<td>Corn stover</td>
<td>93.9±0.2</td>
<td>91.7±0.3</td>
<td>46.9±2.1</td>
<td>0.9±0.003</td>
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</table>

Values are means ± standard deviation of three determinations. Dairy manure and hardwood sawdust mature compost; A mixture of 43% certified organic top soil, 43% of no-till farm soil and 14% sand; Methanogenically active municipal sewage sludge; OFMSW = the organic fraction of municipal solid waste; Dry corn stover grinded to ½ inch.
The soil mixture (300 g dry) was placed in the bottom of a 2-liter (working volume) wide mouth jar (Ball® Corporation, item # 383178). Distilled water was added to bring the moisture content of the mixture to 60% of the moisture holding capacity. PUs (1 g of sample carbon) were then mixed thoroughly into the soil. A solution containing 20 ml of potassium hydroxide (KOH) 0.5N (Fisher Scientific, PA, U.S.) was placed in a cup suspended from the lid of each vessel to trap evolved carbon dioxide (CO₂). Vessels were incubated at 27±1°C in triplicate for a period of 320 days. The amount of CO₂ produced was determined by titrating the KOH solution with 0.25 N hydrochloric acid (Fisher Scientific, PA, U.S.) to a phenolphthalein end-point. Vessels were allowed to sit open for 5 minutes during titration to prevent oxygen concentration in the vessel from falling below 18% during the experiment.

The compostability of PUs under conditions that mimic a commercial scale industrial composting facility was conducted based on ASTM D5338-98 (2003). A more complete description of the methodology to study the compostability of polymeric materials can be found elsewhere (Gómez & Michel, 2013). About 15 g of each PU foam was mixed with 350 g (dry weight) of dairy manure and hardwood sawdust compost inoculum (Table 8). The compost inoculum was collected from a full-scale windrow composting facility at OARDC. The characteristics of the compost mixture are described elsewhere (Michel et al., 2004). Ammonium phosphate (Fisher Scientific, PA, U.S.) was added to the mixture to give a C:N ratio of 20:1 including the carbon content of the PU foam. The initial moisture content of the mixture was adjusted to 60%.

The mixture containing the compost inoculum and PUs were incubated in triplicate for a period of 50 days in 4-liter (working volume) vessels at 55°C (BioCold
Environmental Inc., Fenton, MO, U.S.). The composting vessels were aerated from below at 100±1 ml/min (Grewal et al., 2006). Before entering the vessels air was saturated by bubbling through bottles containing deionized water. The off-gas was then analysed for percent CO₂ using an infrared gas analyzer (Vaisala model GMT 220, range 0 to 20%). CO₂ data was automatically recorded using a Campbell Scientific model 23XL data logger for each vessel every hour.

The anaerobic biodegradation of PUs to biogas, a mixture of predominantly CO₂ and CH₄, under controlled high-solids mesophilic (37±1°C) AD conditions based on ASTM D5511-02. These tests resemble biologically active landfills.

Tests were conducted in triplicate for a period of 105 days in 2-liter (working volume) laboratory-scale batch reactors. An 10 g of PU foam squares were mixed with 450 g wet of methanogenically active sludge obtained from a full-scale (3000m³) AD system treating municipal sewage sludge in January of 2013 (Gómez et al., 2011). This was mixed with 60 g wet of the organic fraction of municipal solid waste (OFMSW) and 30 g dry of dried corn stover (sieved to less than ½ inch particle size) to achieve the desired total solids content for the test (20%). The chemical properties of the organic substrates are shown in Table 8. Ammonium phosphate (Fisher Scientific, PA, U.S.) was added to the mixture to adjust the C:N ratio to a value of 20:1 considering the carbon content of the PU foam.

**Analytical Methods**

The extent of biodegradability of the PUs under soil incubation, composting, and AD conditions was calculated by measuring the average carbon (CO₂ and/or CH₄)
mineralized from each treatment, subtracting the average carbon evolved from the blanks, and dividing this by the total amount of sample carbon added to each treatment. Blanks contained only the mixtures of inoculum and organic substrates (no PU foam added).

A variety of analytical measurements were used to characterize the organic substrates and the PUs that were tested in the experiments. The total solids and volatile solids content were determined by drying the samples to constant weight at 80°C and 500°C, respectively. pH was determined using a pH electrode (TMECC 04.11-A 1:5 slurry method, mass basis). Carbon (TMECC 04.01-A combustion with CO2 detection) and nitrogen content (TMECC 04.02-D oxidation, Dumas method) were determined using a VarioMax N Analyzer (Elementar Americas, NJ, U.S.) by the OARDC STAR Lab.

The volumetric production and the composition of the biogas produced in the AD experiments were analyzed using a water displacement method and gas chromatography as described by Gómez et al. (2011), respectively.

The structural changes of PUs before and after the composting experiments were analyzed by Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), Thermogravimetric Analysis (TGA), and Evolved Gas Analysis Mass Spectrometry (EGA-MS). At termination of each experiment, PUs cuboids were carefully rinsed with DDI water and vacuum dried at 150 in Hg at 40°C for 48 hours (Isotemp® Vaccum Oven model 282A, Fisher Scientific, PA, U.S).

For the SEM analysis, samples were first coated with platinum to a thickness of 0.2kA° using a Hummer® 6.2 sputtering system (Anatech USA, CA, U.S.). SEM images
were taken using a Hitachi S-3500N microscope (Hitachi High Technologies America, Inc., CA, U.S.) with 15Kv electron beam.

FT-IR spectra were recorded using the Spectrum Two™ (Perkin Elmer Inc., MA, U.S.) equipped with a universal attenuated total reflectance accessory (UATR). The FT-IR/UATR was equipped with a diamond crystal allowing for recording the spectra directly on the PUs without sample preparation. FT-IR spectra were averaged over 16 scans from 4000 to 450cm⁻¹ wavenumber with a resolution of 4 cm⁻¹. A background scan of the clean diamond was recorded before scanning the samples. Spectra data were normalized and the baseline was corrected using Perkin Elmer Spectrum software (application version 10.03.07.0112).

Thermogravimetric analyses were carried out using a TA Q 50 Thermogravimetric Analyzer (TA Instruments, DE, U.S.). Thermogravimetric curves were obtained using approximately 2 mg of sample. Temperature was increased at a heating rate of 20 °C min⁻¹ from 50 to 600°C under nitrogen atmosphere at a rate of 60 mL min⁻¹. The Universal Analysis 2000 software was used for curves analyses, version 4.5A (TA Instruments, DE, U.S.).

The evolved compounds during the thermal decomposition of the PUs were characterized by evolved gas analysis-mass spectrometry (EGA-MS) using a multi-shot pyrolyzer (EGA/PY-3030 D, Frontier Lab, Fukushima, Japan) coupled with a GC-MS (GCMS-QP2010 SE, Shimadzu, MD, U.S.) via a UADTM-2.5N column (2.5 m, 0.15 mm i.d., Frontier Lab, Fukushima, JAPAN). Helium was used as the carrier gas at a flow rate of 1 mL min⁻¹ at a split ratio of 30. The column oven temperature was kept at 300 °C. The pyrolyzer temperature was held at 70 °C for 0.5 min, then heated to 600 °C at a rate of 20
°C min⁻¹, and held at 600 °C for 1 min. The mass spectrometer was operated in the electron ionization (EI) mode with m/z scan range of 0–500, and its ion source temperature was kept at 200 °C. The pyrolyzates were identified by comparing their mass spectra with those reported in the NIST library.

**Statistical Analysis**

Three independent replicates were used for each treatment. Analysis of variance (ANOVA) was calculated for the average final cumulative percent of initial carbon loss for each of the studies. Comparisons for all pairs of final cumulative biodegradation means were performed using Student’s t method. All conclusions were based on a significant difference level of α=0.05. The statistical analyses were performed using JMP statistical program version 9 (SAS Institute Inc., SAS Campus Drive, NC, U.S.).
Results and Discussion

Biodegradation studies

The biodegradation in soil experiment was conducted for a period of 320 days (Figure 7a). The highest initial extent of mineralization was obtained with the PUs made from bio-based polyols with 8.46±2.0% during the first 87 days of study (Figure 7a). Increases in the biodegradation extent for the bio-based PUs continued until day 146 reaching 10.9±2.2% (Figure 7a). This was followed by a period of slow mineralization that continued until the termination of the experiment (Figure 7a). Lower initial mineralization extents were observed for the PUs made from petroleum-based and the blend polyols with values ranging between 1 and 2.5% during the first 146 days of experiment (Figure 7a). Final (320 days) cumulative biodegradation values for the PUs made from petroleum, bio-based, and blend polyols reached 1.65±1.4%, 11.2±4.3% and 2.90±1.0%, respectively (Figure 7a). The positive control (cellulose paper) exhibited 65.4±6.0% conversion (Figure 7a).

ANOVA statistical analysis revealed that significant differences (p<0.05) between group means of the PUs cumulative carbon loss during soil incubation existed. Further analysis revealed that the extent of mineralization measured for the PUs made from bio-based polyols was significantly different (p<0.05) from those of the petroleum-based and blend materials. In addition, differences observed between the petroleum-based and the blend PUs were not significant (p>0.05).
Figure 7. Cumulative carbon loss (CO$_2$-C) as percentage of initial carbon (average of three determinations ± cumulative standard error) for PUs made from petroleum, bio-based and blend polyols during 320 days of soil incubation (a), 50 days of composting (b) and 105 days of anaerobic digestion (c). For some data points standard error bars are smaller than markers.
Under composting conditions the initial mineralization extents of the PUs were similar during the first 18 days of study with values ranging between 4 and 8% across all treatments (Figure 7b). This was followed by continuous increases in the mineralization extents of the PUs made from bio-based and blend polyols until day 46 of experiment (Figure 7b). On the other hand, increases in the mineralization extent of the PUs made from petroleum-based polyols were marginal until termination of the experiment (Figure 7b). The final (50 days) cumulative biodegradation values measured for PUs made from petroleum, bio-based, and blend polyols were of 3.60±5.5%, 12.6±6.4% and 16.2±8.0%, respectively (Figure 7b). The positive control (cellulose paper) exhibited 77.2±8.7% conversion during the 50 days period of study (Figure 7b).

SEM images obtained before and after composting showed important changes in the surface of the PUs made from polyols with at least 50% of bio-based content and almost no indication that biodegradation processes occurred in the petroleum-based analogs (Figure 8).

The biodegradation of PUs under anaerobic conditions was conducted over a period of 105 days in batch mode (Figure 7c). Biodegradation during the first 15 days of study was slow across all treatments (Figure 7c). This was followed by increases in the mineralization extent of the PUs made from bio-based and blend polyols between days 22 and 29 of study with values reaching 5.36±1.2 and 6.59±1.4%, respectively (Figure 7c). The mineralization extent of the PUs made from petroleum-based polyols showed a marginal increase until day 67 and remained virtually constant until termination of the study reaching a final cumulative biodegradation of 3.53±3.3% (Figure 7c). Final cumulative carbon loss values for the PUs made from bio-based and blend polyols, and
the positive control were of 8.93±1.8%, 8.46±2.3% and 79.4±5.6%, respectively (Figure 7c). Statistical analyses revealed that differences between group means of the PUs cumulative carbon loss observed during composting were not significant ($p>0.05$). In the same manner, differences observed between group means during AD studies were also not significant ($p>0.05$).

Results of these studies showed differences in the extent of biodegradation for each foam under different incubation schemes. The mineralization extent of the PUs made from petroleum-based polyols was similar under composting and AD conditions. Moreover, a much lower extent was observed during soil incubation for the same material. For the PUs made from bio-based and blend polyols the highest extent in mineralization was observed during composting. This was followed by the AD condition for the PUs made from blend polyols and by soil incubation for the bio-based analog. The lowest mineralization extents for the PUs made from blend and the bio-based polyols were observed during soil incubation and AD, respectively.
Figure 8. Scanning electron microscopy before and after incubation during 50 days of composting for PUs made from petroleum (a: before, b: after), bio-based (c: before, d: after) and blend (e: before, f: after) polyols.
**Thermogravimetric (TGA) analysis**

The thermal decomposition of the PUs made from petroleum-based polyols was mainly characterized by one single-stage weight loss before and after 50 days of composting at 55°C (Figure 9a). This weight loss occurred in the thermal region located between 240 and 400°C (Figure 9a). This drastic thermally-induced single-step weight loss behavior is characteristic of petroleum-based PUs materials (Javni et al., 2000; Zhang & Feng, 2004). Weight losses occurring in temperatures ranging between 150 and 400°C in petroleum-based PUs have been attributed to decomposition of the urethane segments (Petrovic et al., 1994). However, the onset of the thermal decomposition has been reported to occur anywhere between 150 to 260°C and can be influenced by many factors including the types of substituents on the isocyanate and polyol sides (Wirpsza, 1993), and the atmosphere used during thermal decomposition analyses (Javni et al., 2000).

The PUs samples made from bio-based polyols obtained before and after composting were characterized by two major stages of weight loss (Figure 9b). The first stage of thermal decomposition occurred in temperatures ranges of 190 to 320°C and 240 to 340°C for the samples analyzed before and after composting (Figure 9b), respectively. In this region, decomposition of urethane groups in PUs made from vegetable oil-derived polyols has been reported (Cangemi et al., 2006; Guo, 2000; Monteavaro et al., 2005). The major thermal decomposition in the bio-based PUs occurred during the second stage with approximately 50% weight loss for the samples analyzed before and after composting (Figure 9b). This stage occurred in temperature ranges of 340 to 530°C and 360 to 540°C for the samples analyzed before and after composting, respectively (Figure
This two-stage thermal decomposition behavior has been previously reported in PUs made from vegetable oil-derived polyols (Javni et al., 2000). Furthermore, weight losses in these temperature ranges have been attributed to the decomposition of ester links and fatty acid chains in the vegetable oil-derived polyols portion (Cangemi et al., 2006; Claro Neto, 1997) as these have higher stability against thermal decomposition than the urethane segments (Simon et al., 1988).

PUs made from blend polyols show TGA curves similar to those of PUs from bio-based polyols (Figure 9b and c). Two stages of weight losses were observed for samples before and after composting. The first stage in the blend PUs occurred in temperature ranges of 180 to 320°C and 200 to 340°C for the samples obtained before and after composting, respectively (Figure 9c). The second stage occurred in temperature ranges of 350 to 540°C and 370 to 550°C (Figure 9c) for the samples obtained before and after composting, respectively (Figure 9c). By comparing the TGA curves of PUs made from petroleum-based polyols before and after composting, it was found that there were no obvious changes indicating their low degradability. Both PUs made from bio-based and blend polyols showed higher thermal stability after undergoing a composting process. This indicated the occurrence of structural changes during biodegradation processes. Other studies conducted with PUs made from vegetable oil-based and liquefied biomass-derived polyols have also reported shifts to a higher temperature in the overall thermal decomposition curve after undergoing a biodegradation process (Cangemi et al., 2006; Zhang et al., 2012). The increase of their thermal stability of PUs from bio-based and blend polyols after composting could be attributed to a decrease in the content of urethane segments during the composting process (Lu & Larock, 2008).
Figure 9. Thermogravimetric curves of PUs made from petroleum (a), bio-based (b) and blend (c) polyols before and after 50-day composting.

**Evolved gas analysis**

Evolved gas analysis (EGA) was conducted to identify evolved compounds at different stages of the thermal decomposition profile in PUs from petroleum and bio-
based as well as from blend polyols before and after a 50-day composting process. The EGA temperature program was set to be as similar as the one used for the TGA analysis.

The overall thermograms of the petroleum-based PUs showed evolution of compounds (Figure 10a) in temperature ranges similar to decompositions observed during TGA analysis (Figure 9a). Differences between the thermograms of the petroleum-based PU foams before and after composting were also marginal. The thermogram of the PUs made from petroleum-based polyols was characterized by two peaks. The first small peak of evolved compounds was observed in temperature ranges of 100 to 200°C before and after composting. Mass spectra analysis revealed that evolved compounds presented compositional characteristics of aromatic compounds. Moreover, the intensity of evolved compounds in this thermal region was lower for the sample after composting. This reduction could be attributed to the release of volatile compounds from the additives used during the foam preparation process (Zhang et al., 2012). The second peak of evolved compounds in the petroleum-based PUs was observed in temperatures ranging between 220 and 400°C (Figure 10a). Analysis of the compositional characteristics via MS revealed that evolved compounds were originated from polyurethanes via the rupture of urethane bonds. This was in agreement with the results observed during TGA analysis in similar temperature ranges (Figure 9a).
Figure 10. EGA thermograms of PUs made from petroleum (a), bio-based (b), and blend (c) polyols before and after incubation during 50 days of composting.

The thermogram of PUs made from bio-based polyols revealed that important structural changes occurred during the composting process (Figure 10b). As can be seen, the peak in temperatures ranging between 110 and 200°C almost disappeared after the
composting process. The MS analysis revealed that evolved compounds in this thermal region were fatty acid methyl esters (FAMEs), residual compounds which might not be completely consumed in the production of bio-based polyols (Luo et al., 2013). This indicates that residual FAMEs in bio-based polyols were degraded during the composting process. These results could also explain the initial minor weight loss observed in the TGA curve of the uncomposted PUs made from bio-based polyols (Figure 9b). As the increase of heating temperature, another difference of EGA curves in temperatures ranging between 200 and 320°C was observed. The MS analysis revealed that evolved compounds were characteristic of fragments similar to those reported during rupture of the urethane bonds. For the sample analyzed after composting the onset of the peak increased by 30°C (Figure 10b). The third difference in the thermogram of PUs made from bio-based polyols analyzed before and after composting was observed in temperatures ranging between 330 and 540°C. A significant reduction in peak intensity was observed after the composting process. An increase in the onset temperature was also observed for the composted PUs. Compounds evolved in this thermal region showed compositional characteristics of diphenylmethane diamine and fatty acid chains in the polyol side of PUs.

The thermograms of the PUs made from blend polyols was characterized by three peaks for the samples before composting (Figure 10c). The first peak, which was previously attributed to the decomposition of FAMEs-like products, was observed in temperature ranges similar to those reported for PUs made from bio-based polyols (Figure 10b). The peak also disappeared after the composting process in the blend PUs. The second peak in the PUs produced from blend polyols was also attributed to the
decomposition of urethane groups. The third peak in the PUs made from blend polyols, behaved differently in both intensity and onset temperature after composting (Figure 10c). Overall, these results suggest that structural changes in the PUs made from either bio-based or blend polyols occurred during the composting process was mainly due to the biodegradation of FAMEs and ester compounds with fatty acid chains.

**FT-IR analysis**

The structural analysis of PUs made from petroleum and bio-based as well as blend polyols before and after 50 days of composting was performed by FT-IR spectroscopy and the results are shown in Figure 11. The specific location of important bands in the infrared spectra of PUs were assigned according to previous reports (de Haseth et al., 1993; Lobo & Bonilla, 2003). Characteristic PUs at bands of 1721-1722, 1529-1536, 1510-1511, 1281-1306, and 1204-1220 cm\(^{-1}\) were assigned to free urethane carbonyl (C=O) stretching, hydrogen-bonded and free N‒H bending, urethane and ester C–O stretching vibrations, respectively. The band at 2277-2280 cm\(^{-1}\) was assigned to the asymmetric stretching of isocyanate groups.

The band at 2277-2280 cm\(^{-1}\) in the FT-IR spectra of the PUs made from petroleum, bio-based and blend polyols before composting indicated that there were still unreacted isocyanate groups (Figure 11). Moreover, the intensity of this band decreased substantially after composting suggesting that isocyanate groups undergone further reactions during the mineralization study. Reactions may have included formation of additional urethane linkages, and in environments with high moisture content, such as in a composting, these groups can react with water to form urea linkages (Leventis et al., 2003).
Formation of urea groups in the hard segments of the PUs structure could have been one of the factors influencing the behaviors observed during TGA (Figure 9) and EG (Figure 10) analyses as these groups have been reported to be more thermally stable than the urethane ones (Chang et al., 2001).

Slight reductions in the intensity of the bands at 1721, 1529 and 1281 cm$^{-1}$ were observed in the spectra of the PUs made from petroleum-based polyols after composting (Figure 11a). These results suggest that deterioration occurred in the urethane bonds of the PUs structure during composting. Degradation of these bonds due to hydrolytic enzyme activity of microorganisms has also been previously reported to occur in PUs made from petroleum-based polyester polyols (Filip, 1978; Ozsagiroglu et al., 2012). Rupture of these bonds could also be one of the factors influencing the behaviors observed during TGA (Figure 9a) and EGA (Figure 10a) as these are less thermally stable than other major segments in the polymer structure (Lu & Larock, 2008).

The band at 1721, together with the one at 1220 cm$^{-1}$ in the PUs made from petroleum-based polyols, corresponded to ester groups in the polyol segments of the PUs structure. A slight decrease in the C–O stretching intensity of the aromatic ester indicates that degradation of these segments was not the preferred site for microbial attack during composting process. Moreover, biodegradability of these segments has been reported by Kay et al. (1993) and Nakajima-Kambe et al. (1999).
Figure 11. FT-IR spectra of PUs produced from petroleum (a), bio-based (b) and blend (c) polyols before and after incubation during 50 days of composting.
Characteristic bands of the urethane bonds in the bio-based PUs were located at 1722, 1536 and 1512 cm$^{-1}$ (Figure 11b). No substantial decreases were observed except for the 1720 cm$^{-1}$ band (Figure 11b). Furthermore, decreases in the C=O carbonyl bond could have been related to the ester segment of the foam instead. Further analysis revealed that bands assigned to the ester C-O bond (1204 cm$^{-1}$) of the glyceride structure also showed significant decreases in their intensity after the composting process (Figure 11b). These results suggest that rupture of the ester bonds of glyceride structure was the preferred site of microbial attack during degradation of PUs from bio-based polyols in the composting condition. Other studies conducted to evaluate the biodegradability of PUs produced from bio-based polyols including those produced from castor oil (Cangemi et al., 2006; Wang et al., 2008) and soybean oil (Shogren et al., 2004) based have also reported susceptibility of the glyceride-based ester linkages to microbial attack.

The FT-IR spectra of the PUs made from blend polyols before and after composting are shown in Figure 11c. Small decreases in the intensities of the bands assigned to the urethane segments at 1536 (N-H), 1510 (N-H) and 1301 cm$^{-1}$ (urethane C-O) vibrations were observed. Furthermore, obvious decreases were observed in those bands associated with the ester segments at 1722 (C=O) and 1214 (C-O) cm$^{-1}$ stretching vibrations (Figure 11c). These results indicate that ester segments in the PUs made from blend polyols were the preferred site for microbial attack, whereas little degradation occurred in the urethane linkages.
Conclusion

Mineralization studies showed that polyurethane foams from 100% of bio-based derived polyols achieved biodegradation rate different to those observed for the petroleum-based analogs only during 320 days of soil incubation. No significant differences in the extent of cumulative carbon losses between foams from either bio-based or blend polyols and their petroleum-based counterparts were observed during composting and in anaerobic digestion conditions.

For the polyurethane foams analyzed before and after composting, the SEM analysis showed that some surface deterioration occurred in the PUs samples made from bio-based and blend polyols. Although some differences were observed in the TGA curves of the petroleum-based foams analyzed before and after composting, the most prominent structural changes occurred in both polyurethane foams made from bio-based and blend polyols. Further analysis revealed that both urethane and ester-related segments in these two polyurethane foams suffered substantial structural changes during composting. In terms of EGA-MS analyses, the major degradation of PUs made from bio-based and blend polyols during composting was attributed to the decomposition of FAMEs and partial decomposition of glyceride structures in the PUs. In addition, FT-IR analysis showed that little degradation during composting in the petroleum-based PU foam occurred in both urethane and ester segments of the polymer. Studies conducted in the foams made from 100% bio-based polyols revealed that the ester segments (C=O and C-O) of the material were the preferred sites of microbial attack. The PUs made from blend polyols showed some structural changes in the urethane linkages (N-H and C=O)
and degradation was more noticeable in the ester segments (C=O and C-O) of the polymer.
Chapter 4 - Effects of Feedstock Particle Size and Performance of Laboratory and Pilot-scale Anaerobic Digesters Treating Cavitated Sewage Sludge.

Abstract

Treatment and disposal of large amounts of combined sludge produced during primary, secondary and tertiary treatment account for up to 50 to 60% of the total costs of wastewater treatment. While several methods can reduce sludge volumes, AD has the ability to reduce the volume of solids while transforming the organic material into biogas. Whereas raw primary sludge degrades very well in AD systems, secondary sludge is an energy depleted, recalcitrant material and this has led to the development of different pretreatment methods. In this study, the effects of hydrodynamic cavitation on feedstock particle size reductions and performance of anaerobic digesters treating sewage sludge were evaluated. Studies conducted of feedstocks containing mostly primary or secondary sludge showed that cavitation pretreatment was able to achieve substantial reductions in substrate particle size when total solids content ranged between 0.38 and 3.55%. In contrast, reductions in particle size were marginal when a feedstock containing 5.21% total solids was processed at low cavitation process conditions. Laboratory-scale batch AD conducted with feedstocks containing mostly secondary sludge showed that cavitation pretreatment increased total biogas production and volatile solids reduction by
66.8 and 34.2%. However, AD of mostly primary sludge showed little impact of cavitation pretreatment.

**Introduction**

To treat domestic and industrial wastewater, treatment plants use physical, chemical and biological processes. Wastewater is treated until regulatory limits for discharge into the environment are met (Figure 12). Several methods including thickening and dewatering (Turovskii, 2006), incineration (Werther & Ogada, 1999), composting (Carroll et al., 1993), and aerobic (Tilley, 2011) and anaerobic digestion (Gómez et al., 2011) are used to reduce associated volumes of free water and/or solids (Arthurson, 2008).

In general, municipal sewage sludge or biosolids contains approximately 97-99% water and the remaining fraction is composed of organic and inorganic solids (Appels et al., 2008). Conventional wastewater treatment starts with preliminary screening to separate large debris from the influent stream. Suspended solids and floatables are then physically separated from solids using clarifiers during primary treatment (Ødegaard, 1998) the result being a liquid fraction and a solids fraction called primary solids. During secondary treatment, the liquid fraction consisting of colloidal and dissolved organics is introduced into a large stirred tank reactor (s) and biologically treated using aeration and the broad consortium of microorganisms contained in the solids already present in the reactor and known as waste activated sludge (U.S. EPA, 2004). After secondary treatment, the solid and liquid fractions are again separated resulting in secondary sludge and a liquid fraction. During tertiary treatment, additional nutrients and organics are
removed using chemical processes (Shammas et al., 2006). Effluent from tertiary treatment is then disinfected to reduce pathogens before discharge into natural water bodies.

Treatment, handling, transport and disposal of the large amounts of the solids known as combined sludge that is generated during primary, secondary and tertiary treatment accounts for up to 50 to 60% of the total wastewater treatment costs (Davis & Hall, 1997; Spellman, 1997).

![Generalized wastewater treatment process schematic diagram from Rivard & Nagle (1996).](image)

Figure 12. Generalized wastewater treatment process schematic diagram from Rivard & Nagle (1996).

Anaerobic digestion (AD) technology is one of the techniques used for sludge stabilization and organics recycling in modern wastewater treatment plants (Ponsá et al., 2008). AD has the potential to reduce solids by transforming part of the organic material into biogas, a mixture of methane (CH\textsubscript{4}), carbon dioxide (CO\textsubscript{2}) and trace gases (Gómez et al., 2011). Methane is the principal component of natural gas and it is currently used for
electricity production, pipeline quality natural gas, and as a transportation fuel (Chynoweth et al., 2001).

In AD, the performance of the anaerobic reactor is influenced by two main factors; reactor design and operational parameters, and feedstock characteristics (Chynoweth et al., 2001; Gunaseelan, 1997; Rulkens, 2007). In general, wastewater treatment plants use a mixture of primary and secondary sludges produced during primary and secondary treatment as the main feedstocks for the anaerobic digestor (Parkin & Owen, 1986; Show et al., 2012). The feedstock ratio of primary to secondary sludge varies from plant to plant and is affected by the design and efficiency of the overall wastewater treatment process, sludge disposal fates, and the design of the anaerobic digestion system (Vesilind, 1974).

The physical and chemical properties of primary and secondary sludge are very different in characteristics and behavior (Table 1). Raw primary sludge consists of undigested settleable solids with a high content of putrescible organic matter such as food waste, fecal matter, fiber, and floatable solids such as grease (Shuval, 1977). Raw primary sludge degrades very well in anaerobic digestion systems and its conversion to biogas per pound of sludge is greater than for any other municipal sewage sludge combination (ASCE, 2000).
Table 9. Typical physical and chemical and properties of primary and secondary sludge (Tchobanogloous et al., 2003)

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</tbody>
</table>

Secondary sludge, also known as biological sludge, is the byproduct of the biological conversion of suspended and soluble organic materials present in the effluent of primary treatment in aerobic conditions by a controlled population of microorganisms known as activated sludge (U.S. EPA, 2004). Secondary sludge is an energy depleted, recalcitrant material, composed of microbial flocs and exopolymeric substances comprising a mixture of biopolymers, polysaccharides, uronic acids and humic substances, among others (Comte et al., 2007; Wilén et al., 2003; Zhang & Bishop, 2003).

The recalcitrant characteristics of secondary sludge flocs limits the hydrolysis stage of the anaerobic digestion process (Carvajal et al., 2013; Eastman & Ferguson, 1981; Noike, 1992). Therefore, the majority of scientific research is directed toward pretreatments that increase the solubilization and disintegration of the organic materials in biologically derived sludge (Carrère et al., 2010; Mao et al., 2004). To date, most of
the research has been conducted on biological treatments (Ge et al., 2010; Roberts et al., 1999), thermal hydrolysis (Haug et al., 1978; Tanaka et al., 1997), chemical oxidation (Weemaes et al., 2000; Yeom et al., 2002), alkali treatments (Kim et al., 2003; Valo et al., 2004), mechanical collision plate and shear (Muller et al., 2003; Nah et al., 2000), and ultrasound (Pérez-Elvira et al., 2009; Pilli et al., 2011).

Ultrasound pretreatment is one of the most promising pretreatment methods for sludge disintegration. Both laboratory and full-scale results have shown improvements in disintegration rates (Weemaes & Verstraete, 1998), increases in biogas production and volatile solids reduction (Braguglia et al., 2006; Hogan et al., 2004), improved dewaterability (Riera-Franco de Sarabia et al., 2000; Yin et al., 2004), among others. It also is environmentally benign since chemicals are not added to the sludge. However, the advantages of ultrasound pretreatment at full-scale are diminished by its’ high energy consumption (Weemaes & Verstraete, 1998), and difficulties adapting equipment to variable sewage sludge characteristics (Pilli et al., 2011).

Another type of ultrasound pretreatment of biological sludge that is simpler and less costly (Senthil Kumar et al., 2000) is hydrodynamic cavitation. Hydrodynamic cavitation is generated by passing a flowing liquid through a constriction to increase the kinetic energy (velocity) at high pressures. Substantial amounts of energy are then lost in the form of a permanent pressure drop in the liquid and/or at the boundary of the constriction. If the local pressure drop falls below that of the liquid vapor pressure, large numbers of gas or vapor filled cavities are formed. Downstream of the constriction the pressure recovers causing the cavities to violently collapse in a process that takes microseconds (Gogate & Pandit, 2005). The catastrophic collapse of the cavities
generates local temperatures and pressures that rise tremendously (Suslick, 1989; Tiehm et al., 2001). These forces can cause physical and chemical changes in the sludge exposing light organic materials to further anaerobic digestion (Gogate & Kabadi, 2009; Onyeche et al., 2002b).

Currently, there is a great need to develop sewage sludge pretreatment methods that improve biogas production and volatile solids reduction in anaerobic digesters. There is also a need to improve the dewaterability and thickening during wastewater treatment. However, most of the pretreatments to date are not energy efficient, require long retention times and/or use chemicals that could represent an added impact to the environment of the wastewater treatment process. In this sense, hydrodynamic cavitation can offer new possibilities to wastewater treatment facilities to improve their operations and the cost-effectiveness of the overall process. However, there is a lack of information on the effects of hydrodynamic cavitation on sludge disintegration and anaerobic digestion performance.

For these reasons, we hypothesized that hydrodynamic cavitation could improve wastewater treatment operations and provide an effective, energy efficient and non-hazardous method to disintegrate municipal sewage sludge and provide benefits such as better dewaterability of primary sludges, and improved biodegradability of secondary sludges. For this study, a controlled flow cavitation device (patents pending) was evaluated.

The overall objective of this study was to evaluate the ability of hydrodynamic cavitation to reduce the particle size of mixed primary and secondary sewage sludge obtained from three different full-scale wastewater treatment facilities in Ohio, US. In
addition, biomethanization studies were conducted to evaluate the effects of hydrodynamic cavitation on AD process stability and performance.

**Materials and methods**

*Experimental treatments*

Several studies were conducted on different sewage sludge feedstocks pretreated with cavitation technology to evaluate the effects on particle size reduction and reactor performance. Each study had a control loaded with uncavitated feedstock to simulate conventional wastewater treatment operations.

The cavitated feedstocks were pretreated with an under development cavitation unit (patents pending). The cavitation pretreatment consisted of a combination of passes and pressures. A “pass” is defined as the number of events in which the substrate is passed through the cavitation unit. A “pressure” is defined as the pressure drop reached when the substrate is passing through the cavitation unit. Each pass and pressure was denominated with a base value, X, as these are currently trade secrets. The actual increments in X are represented with a numerical value.

At the pilot-scale one treatment was evaluated with no replications (Table 10). Studies were conducted in two identical continuously stirred tank reactors. One reactor was fed uncavitated feedstock and served as the control (Table 10). The treatment reactor was fed cavitated feedstock (Table 10). Each reactor was fed for 50 days.
Table 10. Description of the two pilot-scale experimental treatments. The feedstock was City of Wooster (Ohio, U.S.) Water Pollution Control Plant sewage sludge.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Feedstock&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Passes Condition</th>
<th>Pressure Condition</th>
<th>Hydraulic retention time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70-80 primary + 20-30% secondary sludge</td>
<td>0X</td>
<td>0X</td>
<td>25</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>1X</td>
<td>2X</td>
<td>25</td>
</tr>
</tbody>
</table>

<sup>a</sup>% v/v.

In addition to the continuous feed study, five different batch laboratory-scale studies were conducted to evaluate the effects of cavitation on substrates from different wastewater treatment plants. Two laboratory-scale studies were conducted with City of Wooster sludge. A different study was conducted with City of Rocky River feedstock. The fourth and fifth studies were conducted with City of Lima substrate. A complete description of the experimental treatments evaluated during the laboratory-scale batch studies can be found in Table 11, Table 12 and Table 13. For City of Wooster and City of Rocky River studies each treatment consisted of four independent replicates. The City of Lima laboratory experiment consisted of three (lab-1) and five (lab-2) independent replicates.
Table 11. Description of the City of Wooster batch laboratory-scale experimental treatments. The feedstock was City of Wooster (Ohio, U.S.) Water Pollution Control Plant sewage sludge.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatments</th>
<th>Feedstock&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Collection Date</th>
<th>Passes Condition</th>
<th>Pressure Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>70-80 primary + 20-30% secondary sludge</td>
<td>7-18-2013</td>
<td>0X</td>
<td>0X</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1X</td>
<td>2X</td>
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<td>3</td>
<td></td>
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<td>2X</td>
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<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td>2X</td>
<td>3X</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>70-80 primary + 20-30% secondary sludge</td>
<td>8-16-2013</td>
<td>0X</td>
<td>0X</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1X</td>
<td>2X</td>
</tr>
</tbody>
</table>

<sup>a</sup>% v/v.

Table 12. Description of the City of Rocky River batch laboratory-scale experimental treatments. The feedstock was Rocky River (Ohio, U.S.) Wastewater Treatment Plant sewage sludge.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Feedstock&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Passes Condition</th>
<th>Pressure Condition</th>
<th>Enzymatic Pretreatment&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Thermal Pretreatment&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mostly primary sludge</td>
<td>0X</td>
<td>0X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0X</td>
<td>0X</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td></td>
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<td>0X</td>
<td>+</td>
<td>+</td>
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<td>3</td>
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<td>1X</td>
<td>-</td>
<td>-</td>
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<tr>
<td>4</td>
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<td>1X</td>
<td>-</td>
<td>+</td>
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<td>5</td>
<td></td>
<td>1X</td>
<td>1X</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup>Feedstock was collected on 8-22-2013. <sup>b</sup>Enzymatic pretreatment consisted of a mixture of proteases and cellulases. <sup>c</sup>Thermal pretreatment was applied by means of a hot plate and continuous mixing until substrate reached a temperature of 60°C.
Table 13. Description of the City of Lima batch laboratory-scale experimental treatments. The feedstock was City of Lima (Ohio, U.S.) Water and Wastewater treatment plant sewage sludge.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Feedstock&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Collection date</th>
<th>Passes Condition</th>
<th>Pressure Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Mostly secondary sludge</td>
<td>1-15-2012</td>
<td>0X</td>
<td>0X</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1X</td>
<td>1X</td>
</tr>
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<td>3X</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>20 primary + 80% secondary sludge</td>
<td>9-22-2013</td>
<td>0X</td>
<td>0X</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td>1X</td>
<td>2X</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td>2X</td>
<td>4X</td>
</tr>
</tbody>
</table>

<sup>a</sup>% v/v.

**Feedstocks and inoculum**

Figure 13 shows potential locations for sludge pretreatment. The location where the pretreatment is applied depends on factors including the layout of the wastewater treatment plant, operational parameters, substrate characteristics and the objectives of pretreatment.
Figure 13. Potential locations for sludge pretreatment in conventional wastewater treatment plants obtained from Carrère et al. (2010). T1: on activated sludge, T2: on activated sludge circulation loop, T3: on primary sludge, T4: pretreatment on secondary sludge, T5: on mixed primary and secondary and T6: on anaerobic digester recirculation loop.

Methanogenically active inoculum used for the City of Wooster pilot and laboratory-scale studies was obtained from the full-scale high solids anaerobic digester reactor located at the Ohio Agricultural Research and Development Center collected on 7-18-2013. The chemical and physical properties of this inoculum are shown in Table 14. The feedstock used to operate the pilot-scale study was a mixture of 70-80% primary sludge and 20-30% secondary sludge obtained from the City of Wooster Water Pollution Control Plant (Figure 13-T5). Feedstocks were collected on four different dates (Wooster 7-18-2013, 8-2-2013, 8-16-2013 and 8-30-2013) in order to evaluate the substrate variability of a full-scale wastewater treatment plant. The chemical and physical
properties of these feedstocks are shown in Table 15. City of Wooster laboratory-scale 1 study used the same inoculum as the pilot-scale study (Table 14).

The feedstock used for this study was collected on 7-18-2013 (Table 15) and consisted of a mixture of a mixture of 70% primary and 30% secondary sludges (Figure 13-T5). For the City of Wooster laboratory-scale 2 the inoculum was obtained from the full-scale digester on 8-16-2013. The feedstock used for this study was collected on 8-16-2013 (Table 14). The chemical and physical properties of the feedstocks are shown in Table 15.

Activated inoculum for the Rocky River laboratory-scale study was effluent from the full-scale anaerobic digester reactor located at the Rocky River (Ohio, U.S.) Wastewater Treatment Plant collected on 8-16-2013 (Table 14). The feedstock was primary sludge (Figure 13-T3) collected on 8-16-2013 (Table 15).

Methanogenically active inoculum for the Lima laboratory-scale study was effluent of the full-scale anaerobic digester reactor located at the Lima (Ohio, U.S.) Wastewater Treatment Plant (Table 14). The feedstock was a mixture of 20 primary and 80% secondary (v/v) sludges (Figure 13-T5) collected from the same location on 1-15-2012 and 9-22-2013 (Table 15) for studies 1 and 2, respectively.
Table 14. Initial mean characteristics of the inoculum used for the semi-continuous pilot-scale and batch laboratory-scale studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Chemical and physical properties&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total solids (%)</td>
</tr>
<tr>
<td>City of Wooster pilot&lt;sup&gt;b&lt;/sup&gt; and lab-scale 1</td>
<td>3.09±0.02</td>
</tr>
<tr>
<td>City of Wooster lab-scale 2</td>
<td>2.15±0.03</td>
</tr>
<tr>
<td>City of Rocky River lab-scale</td>
<td>3.40±0.01</td>
</tr>
<tr>
<td>City of Lima lab-scale 1</td>
<td>1.97±0.04</td>
</tr>
<tr>
<td>City of Lima lab-scale 2</td>
<td>2.09±0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± standard deviation of three determinations. <sup>b</sup>Parameters including ammonia nitrogen, alkalinity and total volatile fatty acids were only measured for the pilot-scale study feedstocks.
Table 15. Initial mean characteristics of the feedstocks used for the semi-continuous pilot-scale and batch laboratory-scale studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Collection date</th>
<th>Chemical and physical properties&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Total Solids (% ww)</th>
<th>Volatile solids (% dw)</th>
<th>Total carbon (% dw)</th>
<th>Total nitrogen (% dw)</th>
<th>pH</th>
<th>Alkalinity (mg CaCO&lt;sub&gt;3&lt;/sub&gt; eq kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Total VFAs (mg kg&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>City of Wooster feed 1</td>
<td>7-18-2013</td>
<td></td>
<td>2.34±0.02</td>
<td>79.7±0.2</td>
<td>38.3±1.1</td>
<td>6.73±0.1</td>
<td>7.04±0.01</td>
<td>1956±140</td>
<td>476±15</td>
</tr>
<tr>
<td>City of Wooster feed 2</td>
<td>8-2-2013</td>
<td></td>
<td>2.16±0.02</td>
<td>74.7±1.1</td>
<td>40.2±3.0</td>
<td>6.83±0.04</td>
<td>7.19±0.01</td>
<td>1570±307</td>
<td>218±3</td>
</tr>
<tr>
<td>City of Wooster feed 4</td>
<td>8-30-2013</td>
<td></td>
<td>2.86±0.02</td>
<td>56.7±0.4</td>
<td>45.4±2.5</td>
<td>6.63±0.03</td>
<td>7.09±0.02</td>
<td>1709±97</td>
<td>522±27</td>
</tr>
<tr>
<td>City of Rocky River lab-scale</td>
<td>8-22-2013</td>
<td></td>
<td>5.21±0.04</td>
<td>70.0±0.3</td>
<td>44.7±1.2</td>
<td>3.73±0.03</td>
<td>5.46±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>City of Lima lab-scale 1</td>
<td>1-15-2012</td>
<td></td>
<td>2.01±0.1</td>
<td>52.6±1.0</td>
<td>30.3±1.0</td>
<td>3.95±0.2</td>
<td>7.11±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>City of Lima lab-scale 2</td>
<td>9-24-2013</td>
<td></td>
<td>2.78±0.02</td>
<td>61.5±0.6</td>
<td>32.9±0.2</td>
<td>5.68±0.04</td>
<td>7.21±0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are means ± standard deviation of three determinations. <sup>b</sup>Parameters including ammonia nitrogen, alkalinity and total volatile fatty acids were only measured for the pilot-scale study feedstocks.
Feedstock pretreatment

The cavitated feedstocks were pretreated with under development hydrodynamic cavitation devices constructed based on US patents 5937906, 5971601 and 6035897. In summary, different volumes of feedstock were prepared according to the different experimental treatments after collection from the wastewater treatment plant. Beforehand, the feedstock holding tank was filled at the maximum level. The motor pump was operated until reaching pressured drops in accordance with the different experimental treatments. After pressure drop was reached, samples for the experiments were collected. Samples were immediately transported to the laboratory were the experiments were conducted and one sample was taken for immediate particle size and chemical properties analyses.

Studies conducted with the City of Wooster feedstock were cavitated with a bench-scale unit (device A). Feedstock for the City of Lima study 1 was pretreated with a full-scale cavitation system (device B). City of Lima study 2 feedstock was cavitated with a different pilot-scale device (device C). Experiments conducted with the City of Rocky River feedstocks were conducted with a pilot-scale device (device D). Number of passes were achieved by cavitating a previously cavitated sample at the desired pressure drop (Table 10-Table 13).

For the Rocky River laboratory-scale study, thermal pretreatment was achieved using a hot plate (Fisher Scientific, PA, U.S.). The enzymatic pretreatment consisted of a combination of two enzymes. The first enzyme, AP1 (150,000 u/ml), is a native acid protease enzyme derived from Aspergillus niger (CTE Global Inc., IL, U.S.). The second enzyme, XC150 (150,000 u/ml), is a native acid cellulose enzyme produced from
Trichoderma reesei (CTE Global Inc., IL, U.S.). Homogenous temperature and enzyme distribution was achieved using a constant speed overhead electric lab mixer (Fisher Scientific, PA, U.S.).

**Experimental procedure**

The pilot-scale study was conducted in two identical 30 gallon (working volume) continuously stirred tank reactors in a semi-continuous mode at the Ohio Agricultural Research and Development Center (OARDC) Bioproducts and Bioenergy Research Laboratory (Figure 14). Each reactor was fed once a day and an equal amount of effluent was removed. The stainless steel reactors maintained mesophilic conditions (37±2°C) by means of a built-in hot water jacket (Figure 14). Each pilot-scale digester was directly connected to a drum type wet-test volumetric gas meter (RITTER®, Bochum, Germany) to measure biogas production on a regular basis (Gómez et al., 2011).
The two pilot-scale digesters were inoculated with 25 gallons of methanogenically active sludge from the OARDC full-scale anaerobic digester effluent (Table 14). Once inoculated, both reactors were sealed. Both reactors did not receive feed for three days to allow microorganisms to convert readily available substrates into biogas and to check for proper functioning. After proper function of the two digesters was confirmed, feeding started (day zero) and continued for a period of 50 days. Reactors were fed and effluent collected once a day. The two pilot-scale digesters were fed with feedstocks that were collected on four different dates from the City of Wooster Wastewater treatment plant (Table 15), 7-18-2013, 8-2-2013, 8-16-2013 and 8-30-2013 according to the experimental treatments. The feedstock collected on 7-18-2013 (feed 1) was fed on days zero to 15 of
the study, 8-2-2013 feedstock (2) was fed on days 16 to 23, 8-16-2013 feedstock (3) was fed on day 24 to 41 and 8-30-2013 (feed 4) from day 42 to 50. The reactors organic loading rates were of 0.89, 0.77, 1.06 and 0.78 kilograms of volatile solids per cubic meter per day while the digester were operated with 7-18-2013, 8-2-2013, 8-16-2013 and 8-30-2013 feedstocks, respectively.

Different chemical and physical parameters were measured over the 50 day period to assess the effects of feedstock pretreatment on reactor performance and the stability of the pilot-scale reactors. Biogas production was measured daily. The biogas composition was measured two times every week. Biogas production was also measured every three hours during weeks 3, 5 and 7 of study. For the first two weeks, once a week, effluent obtained from the pilot-scale digesters was analyzed for volatile solids content. Afterwards, these parameters were then measured two times a week until termination of the study. Stability parameters including pH, alkalinity and volatile fatty acids were measured two times every week. The ammonia nitrogen content in the effluent of the pilot-scale reactors was measured during weeks 3, 5 and 7 of study.

The batch laboratory-scale assays were conducted in 2-liter (working volume) reactors (Figure 15). Temperatures were maintained at a mesophilic (37±1°C) range by means of incubators.
For all batch scale experiments 150 g wet of feedstock was mixed with 350 g wet of methanogenically active inoculum. Reactors were then sealed with rubber stoppers, connected to the biogas collection bags and experiments started at day zero. The laboratory-scale studies for City of Wooster 1, 2, Rocky River, Lima 1 and 2 were conducted for 30, 24, 23, 28 and 20 days, respectively.

For the laboratory-scale studies volumetric production of biogas was measured using 1-liter Tedlar® bags to collect off-gas from the reactors. The gas volume was measured on a regular basis by pumping the gas through a drum type wet-test volumetric gas meter as described elsewhere (Gómez et al., 2011).
**Analytical Methods**

A variety of analytical measurements were used to characterize the organic substrates and to monitor process performance during the experiments. The total solids and volatile solids content were determined by drying the samples to constant weight at 80°C and 500°C, respectively.

pH was determined using a pH electrode (TMECC 04.11-A 1:5 slurry method, mass basis).

Carbon (TMECC 04.01-A combustion with CO₂ detection) and nitrogen content (TMECC 04.02-D oxidation, Dumas method) were determined using a VarioMax N Analyzer (Elementar Americas, NJ, U.S.) by the OARDC STAR Lab.

The volumetric production and the composition of the biogas produced was analysed as described by Gómez et al. (2011).

Alkalinity and the total volatile fatty acids content in the sludges was measured using a titration method and gas chromatography as described by Gómez et al. (2011), respectively.

The total and soluble chemical oxygen demand (COD) was measured using the dichromate reactor digestion standard method 5220-D (APHA et al., 1998). COD mercury free vials in a measurable range of 0-1500 mg L⁻¹ were used (CHEMetrics, Inc., VA, U.S.). The total COD samples were prepared in a 200 fold dilution with deionized water. Samples for soluble COD were centrifuged at 13,000 rpm for 20 minutes. Then, the supernatant was filtered to less than 0.2 µm using syringe filters (Fisher Scientific, PA, U.S.).
Particle size distribution analyzes were conducted using a Horiba Laser Diffraction particle Size Analyzer LA 950 (Horiba Instruments Inc., NY, U.S.). For this, an 1g wet of sludge was dispersed in deionized water in the analyzer and 50 µL of Triton X-100 (0.1% in DDI water) surfactant was added to avoid particle agglomeration. Data was then exported to Microsoft Excel for analysis.

For the SEM analysis samples were previously coated with platinum to a thickness of 0.2kA° using a Hummer® 6.2 sputtering system (Anatech USA, CA, U.S.). SEM images were taken using a Hitachi S-3500N microscope (Hitachi High Technologies America, Inc., CA, U.S.) with 15Kv electron beam.

**Statistical Analysis**

Statistical analyses were conducted for the laboratory-scale studies. Four independent replicates were used for each treatment. Analysis of variance (ANOVA) was calculated for biogas production, solids reduction and stability parameters. Comparisons for all pairs of means were performed using Tukey-Kramer HSD analysis. All conclusions were based on a significant difference level of $\alpha=0.05$. The statistical analyses were performed using JMP statistical program version 9 (SAS Institute Inc., SAS Campus Drive, NC, U.S.).
Results and discussion

City of Wooster pilot and laboratory-scale studies

Feedstocks particle size distribution

Figure 16a shows the particle size analysis of City of Wooster mixed sludge feedstock collected on 7-18-2013 (feed 1). Even though this feedstock was processed at five different combinations of cavitation passes and pressures, only the control (0X-0X) and T1 (1X-2X) were used for the pilot and laboratory-scale study. The uncavitated (control) feedstock distribution was characterized by two major peaks with an apex located at 100 and 200 µm (Figure 16a), respectively. The distributions observed for the cavitated feedstocks were characterized by one prominent peak located at 26, 22, 19 and 19 µm for T1 (1X-2X), 1X-3X, 2X-2X and 2X-3X (Figure 16a), respectively. A higher reduction in particle size was achieved with increasing cavitation passes and pressures (Figure 16a). However, after 2X-2X the particle size could not be reduced by more than 19 µm (Figure 16a).

The particle size analysis of City of Wooster mixed sludge feedstocks collected on 8-2-2013 (feed 2) is shown in Figure 16b. Even though this feedstock was processed at four different combinations of cavitation passes and pressures, only the control (0X-0X) and T1 (1X-2X) were used for the pilot-scale study. The uncavitated (control) feedstock distribution was characterized by two major peaks with an apex located at 100 and 200 µm (Figure 16b). The distributions of the cavitated feedstocks were characterized by one major peak located at 22, 34, 22 µm for T1 (1X-2X), 1X-1X and 1X-1.5X (Figure 16b), respectively. A higher reduction in particle size was achieved by
increasing the cavitation pressure drop parameter but not the number of passes (Figure 16b).

Figure 16c shows the particle size analysis of the City of Wooster primary sludge feedstock collected on 8-16-13 (feed 3). This feedstock was used for the pilot-scale study only. The uncavitated (control) feedstock distribution was characterized by two major peaks with apexes located at 100 and 230 µm (Figure 16c). The distribution of the cavitated (T1) feedstock was characterized by one major peak located at 39 µm (Figure 16c).

The results of the particle size analysis performed on the City of Wooster feedstock collected on 8-30-2013 (feed 4) are shown in Figure 16d. The uncavitated feedstock (control) showed only one major peak located at 67 µm (Figure 16d). The cavitated feedstock (T1) also showed only one major peak with its apex centered at 20 µm (Figure 16d).
Figure 16. Particle size analysis of City of Wooster feedstocks 1 (a), 2 (b), 3 (c) and 4 (d) collected on 7-18-2013, 8-2-2013, 8-16-2013 and 8-30-13, respectively.
Pilot-scale study

Pilot-scale semi-continuous flow reactor substrate replacement

The two pilot digesters used during this study were inoculated with 25 gallons of uncavitated active inoculum obtained from the full-scale anaerobic digester located at OARDC (Table 14). The digester was fed with 1 gallon of feedstock and an equal amount of effluent removed every day. Only one digester (T1) was operated with cavitated substrate. The following equation was used to estimate the concentration of cavitated material inside T1 reactor over the 50 day period of study (Figure 17):

\[
\frac{dC_i}{dt} = \frac{C_i F_i - C_r F_o}{V}
\]  

(2)

where \(C_i\) is the fraction of cavitated material entering the digester (100\%), \(C_r\) is the fraction of cavitated in the reactor and effluent (0 when \(t=0\)), \(F_i\) is the inflow rate (1 gallon day\(^{-1}\)), \(F_o\) is the outflow rate (1 gallon day\(^{-1}\)) and \(V\) is the total working volume (25 gallons).

Integration and solution of this equation reveals that approximately 87\% of the material inside the digester was cavitated substrate at the end of the 50 day period of study (Figure 17).
Figure 17. Estimated concentration of cavitated material in the pilot-scale digester operated with cavitated material (Treatment 1 at 1X-2X).

**Particle size distribution inside the digesters**

Figure 18a and Figure 18b show the weekly particle size analysis conducted on the effluent of the control and cavitated (T1) pilot-scale digesters. The week 1 particle size analysis of the effluent of the pilot digesters showed one major peak for both treatments centered at 59 and 45 µm for the control (0X-0X) and T1 (1X-2X), respectively (Figure 18). In the control reactor, operated with uncavitated material (control), the weekly particle size analysis revealed the presence of one major peak located at 59 µm for weeks 2, 4, 5, 6 and 7 and 51 µm for the third week of study (Figure 18a).

For the pilot-digester operated with cavitated feedstock (T1) the weekly particle size analysis conducted on the effluent showed a decrease in size of the particles inside the digester during weeks 3, 4 and 5 of study with values centered at 39, 34 and 30 µm,
respectively (Figure 18b). Particle size then remained constant for the last two weeks of study with values centered at 30 µm (Figure 18b).

Figure 18. Weekly particle size analysis of the effluent of the control digester fed uncavitated feedstock (a) and the treatment reactor fed cavitated feedstocks (b). Feedstocks were collected from the City of Wooster wastewater treatment plant.
**Daily biogas production**

Volumetric biogas production was measured on a daily basis (Figure 19). The initial biogas production for the control (0X-0X) and T1 (1X-2X) was 17.4 and 20.1 L, respectively over a 24 hour period (Figure 19). This was followed by an increase in biogas production until day 5 (Figure 19) reaching the highest values observed over the 50 day period of study (25.9 L for control and 24.3 L for T2).

![Figure 19. Daily biogas production (L) as a function of time for the pilot-scale digesters operated with uncavitated (control) and cavitated (T1) feedstocks over a 50 day period study. Vertical gridlines indicates the time at which the digesters were operated with feedstock 1 (TS: 2.34±0.02%\textsubscript{w}, VS: 79.7±0.2%\textsubscript{d}), feedstock 2 (TS: 2.16±0.02%\textsubscript{w}, VS: 74.7±1.1%\textsubscript{d}), feedstock 3 (TS: 3.55±0.02%\textsubscript{w}, VS: 62.1±0.6%\textsubscript{d}) and feedstock 4 (TS: 2.86±0.02%\textsubscript{w}, VS: 56.7±0.4%\textsubscript{d}).](image)

After the first 5 days of study a decrease in biogas production was observed for both digesters with values ranging between 18.9 and 22.4 L for the control (0X-0X) and between 18.1 and 21.6 for T1 (Figure 19). Biogas production then became more stable.
after day 15 of study (Figure 19). After day 15, both digesters were then operated with feedstock 2 and biogas production values were more stable ranging between 17.1 and 21.6 L for the control (0X-0X) and 17.0 and 21.7 for T1 (Figure 19). During operation with feedstock 2 the differences in biogas production were not substantial (Figure 19).

At day 24 of study reactors were operated with feedstock 3 and small increases in biogas production were observed for both digesters (Figure 19). During this period biogas production for the pilot digester operated with uncavitated feedstock (control) remained stable with values ranging between 19.1 and 21.7 L (Figure 19). This was similar to the reactor operated with cavitated feedstock (T1) in which biogas production values ranged between 18.4 and 21.5 L (Figure 19).

For the last 8 days of study the digesters were operated with feedstock 4 (Figure 19). The pilot digester operated with cavitated feedstock (T1) continued with the decrease in biogas production observed in day 38 of study with final values ranging between 13.3 and 15.6 L (Figure 19). A decrease in biogas production for the pilot digester operated with uncavitated material was observed until day 43 of study with final values ranging between 14.8 and 16.4 L (Figure 19). Biogas production for both digesters was similar during the last 4 days of study (Figure 19).

**Average weekly biogas production**

Results of the daily biogas productions were used to calculate the average weekly biogas productions. Results are averages of seven determinations. The initial average weekly biogas production for the control pilot digesters operated with uncavitated (control) and cavitated feedstocks (T1) were 21.5±3.5 L d⁻¹ for both digesters (Figure 20).
However, this difference was not substantial. A decrease in the weekly biogas production was observed for both digesters with values reaching 21.0±1.4 and 20±2.0 L d⁻¹ for the control (0X-0X) and T1 (Figure 20) during the second week of study, respectively. Moreover, no substantial differences were observed between week 1 and week 2 for both digesters (Figure 20). The decrease observed in week two continued until week three of study with approximately 18±1 L d⁻¹ for both digesters (Figure 20). However, the observed decrease was not substantially different between digesters when compared to the two previous weeks of study (Figure 20).

After the third week of study an increase in the average biogas production was observed for both digesters (Figure 20). The average values measured during week 4 reached 19.5±1.3 and 19.8±1.1 L d⁻¹ for the control (0X-0X) and T1 (0X-0X), respectively (Figure 20). This conduct continued for the following two weeks while both digesters were operated with feedstock 3 and observed values were similar to those measured during the first week of study (Figure 20). Moreover, a decrease in biogas production was observed during the final week of study when both digesters were operated with feedstock 4 with values of 17.7±2.4 and 16.8±2.0 L d⁻¹ for the control and T1, respectively (Figure 20).
Figure 20. Average weekly biogas production (L d⁻¹) as a function of time for the pilot-scale digesters operated with uncavitated (control) and cavitated (T1) feedstocks. Values are averages of seven determinations ± standard deviations. Vertical gridlines indicates the time at which the digesters were operated with feedstock 1 (TS: 2.34±0.02%ₚ, VS: 79.7±0.2%ₚ), feedstock 2 (TS: 2.16±0.02%ₚ, VS: 74.7±1.1%ₚ), feedstock 3 (TS: 3.55±0.02%ₚ, VS: 62.1±0.6%ₚ) and feedstock 4 (TS: 2.86±0.02%ₚ, VS: 56.7±0.4%ₚ).

**Average weekly biogas production rates**

Results of the daily biogas productions were used to calculate the average weekly biogas productions rates. Results are averages of seven determinations. The initial average weekly biogas production rate for the pilot digesters operated with uncavitated (control) and cavitated (T1) was of 0.25 L L⁻¹ d⁻¹ for both treatments (Figure 21). This was followed by a decrease in biogas production rates for both digesters during weeks 2 and 3 of study with values reaching as low as 0.21±0.01 and 0.20±0.01 L L⁻¹ d⁻¹ for the control and T1, respectively (Figure 21). An increase was observed after the third week of study for both digesters (Figure 21).
This increase continued until the fifth week of study reaching values of 0.24±0.01 and 23±0.01 L L⁻¹ d⁻¹ for the control and T1, respectively (Figure 21). This conduct was followed by a decrease in production rates that started during week 6 and continued until the last week of study with final values measured at 0.20±0.03 and 0.19±0.02 for the control (0X-0X) and T1 (1X-2X), respectively (Figure 21). None of the differences observed during each week were substantial between both digesters. However, for the digester operated with cavitated feedstock (T1) the final average weekly biogas production rate was substantially different than those measured during the previous two weeks (Figure 21).

![Figure 21. Average weekly biogas production rates (L L⁻¹ d⁻¹) as a function of time for the pilot-scale digesters operated with uncavitated (control) and cavitated (T1) feedstocks. Values are averages of seven determinations ± standard deviations. Vertical gridlines indicates the time at which the digesters were operated with feedstock 1 (TS: 2.34±0.02%w, VS: 79.7±0.2%d), feedstock 2 (TS: 2.16±0.02%w, VS: 74.7±1.1%d), feedstock 3 (TS: 3.55±0.02%w, VS: 62.1±0.6%d) and feedstock 4 (TS: 2.86±0.02%w, VS: 56.7±0.4%d).]
Average cumulative hourly biogas production

The average hourly biogas production was measured during two different days for weeks 3, 5 and 7 of study for (Figure 22a). For the first week of study the average cumulative hourly biogas productions were slightly higher for the uncavitated (control) at different time intervals over one day of measurements (Figure 22a). Moreover, none of the values measured were substantially different among the two treatments (Figure 22a). The final cumulative values reached 19.0±2.0 and 17.9±1.1 L for T1 and T2, respectively (Figure 22a). The hourly measurements show that the reactors did not deplete the added feedstocks in less than 24 hours, or indicate that there was a difference in the rates for the control and treatment reactors.

During week 5 of study no substantial differences were observed between the digester operated with uncavitated (control) and cavitated (T1) feedstocks (Figure 22b) measured at different time intervals. The week 1 average final cumulative hourly biogas production reached 21.1±1.1 and 21.2±0.4 L for the control and T1 during the 24 hours of measurements, respectively (Figure 22b).

During the last week of study the average cumulative hourly biogas production reached only 17.2±3.3 and 15.6±1.4 L for the control and T1, respectively (Figure 22c). No substantial differences were observed between treatments for biogas productions over one day of measurements for the same week (Figure 22c).
Figure 22. Average hourly cumulative biogas production (L) for weeks 3 (a), 5(b) and 7 (c) of study for the pilot-scale digesters operated with uncavitated (T1) and cavitated (T2) feedstocks. Values are averages of 2 determinations per week per treatment ± standard deviations. For some data points standard deviation bars are smaller than markers.
**Methane content in the biogas**

The initial average weekly concentration of methane (CH₄) in the biogas for the pilot digesters operated with uncavitated (control) and cavitated (T1) was of 66±1% across treatments (Figure 23). This was followed by a 1 to 2% decrease in concentration for both digesters for weeks 2 and 3 of study (Figure 23). However, differences in the first three weeks of study were not substantially different among treatments. During the fourth week of study increases were observed for both digesters reaching values of 67.5±3.2 and 66.4±1.5% for the control and T1, respectively (Figure 23). This conduct continued over the last three weeks of study. Overall the methane content in the biogas ranged between 64 and 68% across treatments and differences were not substantial (Figure 23).
Figure 23. Average weekly variation in the methane content (%) in the biogas as a function of time for the pilot-scale digesters operated with uncavitated (control) and cavitated (T1) feedstocks. Values are averages of seven determinations ± standard deviations. Vertical gridlines indicates the time at which the digesters were operated with feedstock 1 (TS: 2.34±0.02%w, VS: 79.7±0.2%d), feedstock 2 (TS: 2.16±0.02%w, VS: 74.7±1.1%d), feedstock 3 (TS: 3.55±0.02%w, VS: 62.1±0.6%d) and feedstock 4 (TS: 2.86±0.02%w, VS: 56.7±0.4%d). For some data points standard deviation bars are smaller than markers.

Average weekly methane yield

The initial average weekly methane yield for the pilot digesters operated with uncavitated (control) and cavitated (T1) was of 190±3 LCH₄ kgVS⁻¹_added across treatments (Figure 24). Methane yield values measured for the second and third week of study were slightly lower than those observed for the first week of study (Figure 24). However, differences among treatments were not substantial during the first three weeks of study.

An increase in the methane yield was observed during the fourth week of study and continued during weeks 5 and 6 reaching values of 187±8 and 179±8 LCH₄ kgVS⁻¹.
The lowest methane yields observed during this pilot study were measured during the last week of study with values of 161±22 and 153±12 L CH$_4$ kg VS$^{-1}$ added for the control and T1, respectively (Figure 24). The final methane yield measured for the cavitated (T1) digester was substantially lower than those observed during weeks 5 and 6 of study for the same digester (Figure 24).

Figure 24. Average weekly methane yield (L CH$_4$ kg VS$^{-1}$ added) as a function of time for the pilot-scale digesters operated with uncavitated (control) and cavitated (T1) feedstocks. Values are averages of seven determinations ± standard deviations. Vertical gridlines indicates the time at which the digesters were operated with feedstock 1 (TS: 2.34±0.02%$\_w$, VS: 79.7±0.2%$\_d$), feedstock 2 (TS: 2.16±0.02%$\_w$, VS: 74.7±1.1%$\_d$), feedstock 3 (TS: 3.55±0.02%$\_w$, VS: 62.1±0.6%$\_d$) and feedstock 4 (TS: 2.86±0.02%$\_w$, VS: 56.7±0.4%$\_d$). For some data points standard deviation bars are smaller than markers.

**Average weekly volatile solids reduction**

The average weekly volatile solids (VS) reductions for the pilot digesters operated with uncavitated (control) and cavitated (T1) feedstocks are shown in Figure 25. Values
for weeks 1 and 2 have no replications. The initial VS reductions for the control and T1 were of 50.7 and 54.3, respectively. This conduct continued until week 3 of study when reduction values went as low as 43.0±13 and 41±13% for the control and T2, respectively (Figure 25). Reduction values increased until week five and continued until week 6 reaching values of 52.8±8 and 49.9±6%. No substantial differences were observed during weeks 3 and 7 of study across weeks and between treatments when measurements were conducted in duplicates.

Figure 25. Average weekly volatile solids reduction (%) as a function of time for the pilot-scale digesters operated with uncavitated (control) and cavitated (T1) feedstocks. Values are averages of two determinations from weeks 3 to 7 only ± standard deviations. Vertical gridlines indicates the time at which the digesters were operated with feedstock 1 (TS: 2.34±0.02%w, VS: 79.7±0.2%d), feedstock 2 (TS: 2.16±0.02%w, VS: 74.7±1.1%d), feedstock 3 (TS: 3.55±0.02%w, VS: 62.1±0.6%d) and feedstock 4 (TS: 2.86±0.02%w, VS: 56.7±0.4%d). For some data points standard deviation bars are smaller than markers.
Average weekly pH

The average weekly pH inside the pilot digesters operated with uncavitated (T1) and cavitated (T2) feedstocks is shown in Figure 26. The initial pH values were measured at 7.65±0.06 and 7.64±0.003 for the control and T1, respectively (Figure 26). This conduct followed over the 50 day period of study with final values measured at 7.52±0.02 and 7.50±0.06 for the control and T1, respectively (Figure 26). Differences among weeks and between treatments did not appear to be substantial over the 7 week period of study.

![Figure 26. Average weekly pH as a function of time inside the pilot-scale digesters operated with uncavitated (control) and cavitated (T1) feedstocks. Values are averages of two determinations ± standard deviations. Vertical gridlines indicates the time at which the digesters were operated with feedstock 1 (TS: 2.34±0.02%\textsubscript{w}, VS: 79.7±0.2%\textsubscript{d}), feedstock 2 (TS: 2.16±0.02%\textsubscript{w}, VS: 74.7±1.1%\textsubscript{d}), feedstock 3 (TS: 3.55±0.02%\textsubscript{w}, VS: 62.1±0.6%\textsubscript{d}) and feedstock 4 (TS: 2.86±0.02%\textsubscript{w}, VS: 56.7±0.4%\textsubscript{d}). For some data points standard deviation bars are smaller than markers.](image-url)
Average weekly total volatile fatty acids concentration

The average weekly total volatile fatty acids inside the pilot digesters operated with uncavitated (control) and cavitated (T1) feedstocks are shown in Figure 27. The initial VFAs values were measured at 43±13 and 66±32 mg kg\(^{-1}\) for the control and T1, respectively (Figure 27). This was followed by a decrease on T1 reaching a value similar to that of observed for the control (Figure 27). During the third week of study, while both digesters were operated with feedstock 3 an increase was observed for both digesters that continued until week 4 of study reaching values of 83±5 and 58±6 mg kg\(^{-1}\) for the control and T1, respectively (Figure 27). During this week differences in VFAs inside the digesters were substantial across treatments. After four weeks of study VFAs decreased for both treatments with final values of 58±17 and 38±8 mg kg\(^{-1}\) for the control and T1, respectively (Figure 27).
Figure 27. Average weekly total volatile fatty acids (mg kg\(^{-1}\)) as a function of time inside the pilot-scale digesters operated with uncavitated (control) and cavitated (T1) feedstocks. Values are averages of two determinations ± standard deviations. Vertical gridlines indicates the time at which the digesters were operated with feedstock 1 (TS: 2.34±0.02\%\(\text{w}\), VS: 79.7±0.2\%\(\text{d}\)), feedstock 2 (TS: 2.16±0.02\%\(\text{w}\), VS: 74.7±1.1\%\(\text{d}\)), feedstock 3 (TS: 3.55±0.02\%\(\text{w}\), VS: 62.1±0.6\%\(\text{d}\)) and feedstock 4 (TS: 2.86±0.02\%\(\text{w}\), VS: 56.7±0.4\%\(\text{d}\)).

**Average weekly alkalinity concentration**

The average weekly alkalinity concentrations inside the pilot digesters operated with uncavitated (control) and cavitated (T1) feedstocks are shown in Figure 28. The initial alkalinity values were measured at 7440±83 and 7569±118 mgCaCO\(_3\)eq kg\(^{-1}\) for the control and T1, respectively (Figure 28). A decrease in alkalinity was observed in week 2 of study for both digesters that continued until week 4 reaching alkalinity values of 5487±78 and 5495±187 mgCaCO\(_3\)eq kg\(^{-1}\) for the control and T1, respectively (Figure 28). After week 4 alkalinity values remained stable until the last week of study with final values measured at 6093±36 and 6094±54 mgCaCO\(_3\)eq kg\(^{-1}\) for the control and T1,
respetively (Figure 28). Differences in alkalinity concentrations were not substantial across treatments for the seven weeks of study. However, weekly alkalinity values were substantially different from week to week.

![Graph showing average weekly alkalinity concentrations over 8 weeks for different feedstocks.](image)

**Figure 28.** Average weekly alkalinity (mgCaCO$_3$ eq kg$^{-1}$) concentrations inside the pilot-scale digesters operated with uncavitated (control) and cavitated (T1) feedstocks. Values are averages of two determinations ± standard deviations. Vertical gridlines indicates the time at which the digesters were operated with feedstock 1 (TS: 2.34±0.02%$\text{w}$, VS: 79.7±0.2%$\text{d}$), feedstock 2 (TS: 2.16±0.02%$\text{w}$, VS: 74.7±1.1%$\text{d}$), feedstock 3 (TS: 3.55±0.02%$\text{w}$, VS: 62.1±0.6%$\text{d}$) and feedstock 4 (TS: 2.86±0.02%$\text{w}$, VS: 56.7±0.4%$\text{d}$).

For some data points standard deviation bars are smaller than markers.

**Laboratory-scale study 1**

The feedstock used for this study was City of Wooster feedstock 1 collected on 7-18-2013 (Table 15). The particle size analysis for this feedstock is discussed in City of Wooster particle size distribution (Figure 16). The AD study was conducted for a period of 30 days under batch mode.
Cumulative biogas production

Cumulative biogas productions across treatments during the first 3 days of study ranged from 345 and 390 ml (Figure 29). This rate was similar over the first 12 days of study (Figure 29). After day 12, higher cumulative biogas productions were observed for the control (0X-0X) and T1 (Figure 29). As shown in Figure 29 a majority of the biogas was produced during the first 19 days of study. The final cumulative biogas production values for the control (0X-0X) and treatments T1 (1X-2X), T2 (1X-3X), T3 (2X-2X) and T4 (2X-3X) were 1718±22, 1681±20, 1616±160, 1570±107 and 1564±78 ml, respectively (Figure 29). Statistical analyses conducted on individual sampling events revealed that differences between group means were not significant (p>0.05).

![Figure 29. Cumulative biogas production (ml) as a function of time. Values are averages of four determinations ± standard deviations of biogas produced from the mixture of feedstock and inoculum. For some data points standard deviation bars are smaller than markers.](image)
Biogas production rates

The initial biogas production rates across treatments ranged between 0.42 and 0.45 ml ml\(^{-1}\)d\(^{-1}\) (Figure 30). The highest initial production rate was measured in the control reactor (0X-0X) with 0.45±0.01 followed by T1 (1X-2X) with 0.44±0.01 ml ml\(^{-1}\)d\(^{-1}\) ml ml\(^{-1}\)d\(^{-1}\) (Figure 30). A drop in biogas productions rates was observed for all treatments during day 5 of study with values ranging between 0.18 and 0.19 ml ml\(^{-1}\)d\(^{-1}\) (Figure 30). During day 7 of study biogas production rates remained constant at 0.19 ml ml\(^{-1}\)d\(^{-1}\) for T2 (1X-3X), T3 (2X-2X) and T4 (2X-3X) (Figure 30). Lower rates were observed for the control (0X-0X) and T1 (1X-2X) for the same day. After day 7 of study production rates continued to decrease until reaching final values as low as 0.01 ml ml\(^{-1}\)d\(^{-1}\) across treatments (Figure 30). Statistical analyses conducted on individual sampling events revealed that differences between group means were not significant (p>0.05).
Figure 30. Biogas production rates (ml ml⁻¹d⁻¹) as a function of time. Values are averages of four determinations ± standard deviations of biogas produced from the mixture of feedstock and inoculum. For some data points standard deviation bars are smaller than markers.

**Methane content in the biogas**

Initial methane content in the biogas across treatments was 69.2±0.1% (Figure 31). A decrease of 3 to 4% in methane content was observed for all treatments during day 7 of study (Figure 31). Methane concentrations then remained constant during the last 23 days of study with measured values ranging between 66 and 69% for all treatments.

Statistical analyses conducted on individual sampling events revealed that differences between group means were not significant (p>0.05). Moreover, values measured during day 7 were significantly different (p<0.05) than those measured during day 5 within treatments.
Figure 31. Methane content in the biogas (%) as a function of time. Values are averages of four determinations ± standard deviations. For some data points standard deviation bars are smaller than markers.

**Methane yield**

The overall yield achieved for the control (0X-0X), T1 (1X-2X), T2 (1X-3X), T3 (2X-2X) and T4 (2X-3X) were of 115±1, 113±1, 106±11, 106±7 and 106±5 mlCH₄ gVS⁻¹ added, respectively (Figure 32). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Figure 32. Overall methane yield (mlCH$_4$ gVS$_{-1}$ added). Values are averages of four determinations ± standard deviations.

_Volatile solids reduction_

The overall volatile solids reduction achieved for the control (0X-0X), T1 (1X-2X), T2 (1X-3X), T3 (2X-2X) and T4 (2X-3X) during the 30 day period of study was of 34.7±3.4, 31.9±4.0, 27.5±7.0, 36.1±1.0 and 36.7±6.0%, respectively (Figure 33). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Figure 33. Overall volatile solids reduction (%). Values are averages of four determinations ± standard deviations.

**Final pH**

The final average pH for the control (0X-0X), T1 (1X-2X), T2 (1X-3X), T3 (2X-2X) and T4 (2X-3X) during the 30 day period of study was of 7.52±0.02, 7.52±0.004, 7.50±0.01, 7.51±0.02 and 7.49±0.01, respectively (Figure 34). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Figure 34. Final pH. Values are averages of four determinations ± standard deviations.

**Laboratory-scale study 2**

The feedstock used for this study was City of Wooster feedstock 3 collected on 8-16-2013 (Table 15) and the highest content of total solids out of the four feedstocks collected from the same location. The particle size analysis for this feedstock is discussed in City of Wooster particle size distribution section (Figure 16). The AD study was conducted for a period of 24 days under batch mode.

**Cumulative biogas production**

Cumulative biogas productions across treatments were very similar during the entire duration of the study (Figure 35). The final cumulative biogas production values for the control (0X-0X) and T1 (1X-2X) were of 1289±984 and 1267±14 ml, respectively.
(Figure 35). Statistical analyses conducted on individual sampling events revealed that differences between group means were not significant (p>0.05).

![Figure 35. Cumulative biogas production (ml) as a function of time. Values are averages of four determinations ± standard deviations of biogas produced from the mixture of feedstock and inoculum. For some data points standard deviation bars are smaller than markers.](image)

**Biogas production rates**

The initial biogas production rates for the control (0X-0X) and T1 (1X-2X) were of 0.36±0.02 and 0.37±0.01 ml ml⁻¹d⁻¹ (Figure 36). The highest rates were observed during day 2 of study with values of 0.99 ml ml⁻¹d⁻¹ across treatments (Figure 36). Statistical analyses conducted on individual sampling events sampling point revealed that differences between group means were not significant (p>0.05).
Figure 36. Biogas production rates (ml ml⁻¹ d⁻¹) as a function of time. Values are averages of four determinations ± standard deviations of biogas produced from the mixture of feedstock and inoculum. For some data points standard deviation bars are smaller than markers.

Methane content in the biogas

Initial methane content in the biogas in both the treatment and control was of 60.5±0.3% (Figure 37). A 5% increase was observed for both treatments during the following 8 days of study (Figure 37). The final methane content in the biogas for the control (0X-0X) and T1 (1X-2X) reached the highest observed values with 71.8±4.0 and 72.0±2.7%, respectively (Figure 37). Statistical analyses conducted on individual sampling events revealed that differences between group means were not significant (p>0.05).
Figure 37. Methane content in the biogas (%) as a function of time. Values are averages of two determinations ± standard deviations. For some data points standard deviation bars are smaller than markers.

**Methane yield**

The overall yield achieved for the control (0X-0X) and T1 (1X-2X) were of 94±5 and 94±2 mlCH4 gVS\(^{-1}\)_added, respectively (Figure 38). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Figure 38. Overall methane yield (mlCH$_4$ gVS$^{-1}$ added). Values are averages of four determinations ± standard deviations.

**Volatile solids reduction**

The overall volatile solids reduction achieved for the control (0X-0X) and T1 (1X-2X) during the 24 day period of study was of 19.8±0.6 and 18.7±0.9%, respectively (Figure 39). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Figure 39. Overall volatile solids reduction (%). Values are averages of four determinations ± standard deviations.

**Final pH**

The final average pH for the control (0X-0X) and T1 (1X-2X) during the 24 day period of study were $7.67 \pm 0.04$ and $7.63 \pm 0.01$, respectively (Figure 40). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Figure 40. Final pH. Values are averages of two determinations ± standard deviations.

City of Rocky River batch laboratory-scale study

Feedstock particle size distribution

Figure 41 shows the particle size analysis of the Rocky River feedstock. The control (0X-0X) feedstock distribution was characterized by two major peaks with apexes located at 15 and 77 µm (Figure 41). This was similar to the distribution in the cavitated and uncavitated treatments (Figure 41). Moreover, the peaks were centered at 13 and 68 µm (Figure 41). The AD experiment was conducted over a 23 day period under batch mode.
Figure 41. Particle size analysis of City of Rocky River feedstock.

**Cumulative biogas production**

Cumulative biogas productions are shown in Figure 42. At day 0.3 of study cumulative biogas productions reached values ranging between 209 and 305 ml. The highest initial biogas productions were observed for the control (0X-0X) and T3 (1X-1X) with 370±3 and 395±6 ml, respectively (Figure 42). Statistical analyses revealed that significant differences (p<0.05) in cumulative biogas productions existed between treatment means at 0.3 day of study. Tukey-Kramer HSD analysis revealed that the cumulative biogas production of the control (0X-0X) and T3 (1X-1X) were significantly different from each other, and from the other treatments.

The conduct observed at day 0.3 continued until day 1.3 of study (Figure 42). At day 1.3 of the highest cumulative biogas production was observed for T3 (1X-1X) reaching a value of 1645±4 ml (Figure 42). For the control (0X-0X), T1 (0X-0X+T), T2
(0X-0X+T+E), T4 (1X-1X+T) and T5 (1X-1X+T+E) biogas productions at day 1.3 reached 1516±14, 1496±31, 1517±14, 1474±27 and 1478±6, respectively (Figure 42). Statistical analyses revealed that differences between group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that the value measured for T3 (1X-1X) was significantly different from the other five treatments.

During day 1.7 of study the highest cumulative biogas production was observed for T4 (1X-1X) reaching a value of 2123±15 ml (Figure 42). For the control (0X-0X), T1 (0X-0X+T), T2 (0X-0X+T+E), T4 (1X-1X+T) and T5 (1X-1X+T+E) biogas productions at day 1.7 reached 1919±27, 1983±108, 2036±30, 1989±29 and 1991±6 ml, respectively (Figure 42). Statistical analyses revealed that differences between group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that the value measured for T4 (1X-1X) was significantly different from the other five treatments.

During day 2.1 of study T3 (1X-1X) continued with the highest cumulative biogas production reaching a value of 2522±22 ml (Figure 42). Cumulative biogas productions measured for the control (0X-0X), T1 (0X-0X+T), T2 (0X-0X+T+E), T4 (1X-1X+T) and T5 (1X-1X+T+E) reached 2256±25, 2396±144, 2478±38, 2423±39 and 2419±9 ml, respectively (Figure 42). Statistical analyses revealed that differences between group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that the values measured for the control (0X-0X) were not significantly different only from T1 (0X-0X+T).

This conduct continued until day 3.9 when the cumulative biogas production of the control (0X-0X) was similar to those measured for T1 (0X-0X+T), T2 (0X-0X+T+E), T4 (1X-1X+T) and T5 (1X-1X+T+E) with values ranging between 3807 and 3955 ml.
The highest cumulative biogas production was measured for T3 (1X-1X) with 4056±29 ml (Figure 42). Statistical analyses revealed that differences in group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that the only significant differences were observed between the control (0X-0X) and T3 (1X-1X).

During day 5.9 of study the highest cumulative biogas productions were measured for the control (0X-0X) and T3 (1X-1X) with 4428±58 and 4507±50 ml, respectively (Figure 42). This was followed by T1 (0X-0X+T) and T5 (1X-1X+T+E) with 4321±165 and 4397±130 ml, respectively (Figure 42). The lowest values were measured for T2 (0X-0X+T+E) and T4 (1X-1X+T) reaching biogas productions of 4232±73 and 4211±38 ml, respectively (Figure 42). Statistical analyses revealed that differences in group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that T2 (0X-0X+T+E) and T4 (1X-1X+T) were significantly different from other treatments but not from each other.

At termination of the study cumulative biogas productions reached 5240±193 for the control and 5031±241, 4871±113, 5260±55, 4850±38 and 5122±155 ml for treatments T1 (0X-0X+T), T2 (0X-0X+T+E), T3 (1X-1X), T4 (1X-1X+T) and T5 (1X-1X+T+E), respectively (Figure 42). Statistical analyses revealed that differences in group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that T2 (0X-0X+T+E) and T4 (1X-1X+T) were significantly different from other treatments but not from each other.
Figure 42. Cumulative biogas production (ml) as a function of time. Values are averages of four determinations ± standard deviations of biogas produced from the mixture of feedstock and inoculum. For some data points standard deviation bars are smaller than markers.

**Biogas production rates**

Biogas productions rates are shown in Figure 43. At day 0.3 of study the highest initial biogas productions rates were observed for the control (0X-0X) and T3 (1X-1X) with 2.73±0.04 and 2.91±0.04 ml ml⁻¹d⁻¹, respectively (Figure 43). The lowest rates were observed for T1 (0X-0X+T) and T4 (0X-0X+T) with 2.20±0.1 and 2.33±0.06 ml ml⁻¹d⁻¹, respectively (Figure 43). Statistical analyses revealed significant differences (p<0.05) in biogas production rates between treatment means at 0.3 day of study. Tukey-Kramer HSD analysis revealed that the cumulative biogas production of the control (0X-0X) and T3 (1X-1X) were significantly different, and different from the other treatments. T1 (0X-0X+T) was also significantly different from the other treatments.
At day 1 the lowest and highest values were measured for the control (0X-0X) and T1 (0X-0X+T) with 2.15±0.05 and 2.34±0.02 ml ml⁻¹ d⁻¹, respectively (Figure 43). Biogas production rates for treatments 3 to 6 ranged between 2.20 and 2.31 ml ml⁻¹ d⁻¹ (Figure 43). Statistical analyses revealed that differences in group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that the rate measured for the control (0X-0X) was significantly different from all the ones of all other treatments.

A sharp increase in biogas production rates was observed for all treatments during day 1.3 of the study (Figure 43). Moreover, the control had the lowest biogas production (Figure 43). Rates for the treatments ranged between 3.01 and 3.22 ml ml⁻¹ d⁻¹. This trend continued until day 4. Statistical analyses conducted until day 4 revealed that differences in group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that differences were significant between the control (0X-0X) and all other treatments.

At day 3.9 of study the highest measured biogas production rate was for the control (0X-0X) with 1.67±0.02 ml ml⁻¹ d⁻¹ (Figure 43). Rates observed for the other treatments ranged between 1.06 and 1.40 ml ml⁻¹ d⁻¹. Statistical analysis revealed that differences between group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that the control (0X-0X) was not significantly different only from T3 (1X-1X). Control (0X-0X) biogas production rates remained the highest until day 10 of study. From day 10 to 23, rates continue to decrease reaching final values ranging between 0.02 and 0.03 ml ml⁻¹ d⁻¹ (Figure 43). Statistical analyses revealed that differences between group means were not significant (p>0.05).

Overall, pretreated feedstocks T1 (0X-0X+T), T2 (0X-0X+T+E), T3 (1X-1X), T4 (1X-1X+T) and T5 (1X-1X+T+E) had higher biogas production rates of 16.0±6, 19.6±11,
12.3±5, 19.1±11 and 17.3±9% than the control (0X-0X) between days 1 and 3 of study. Statistical analyses conducted on the average increase revealed that differences between the treatment group means of T1 (0X-0X+T), T2 (0X-0X+T+E), T3 (1X-1X), T4 (1X-1X+T) and T5 (1X-1X+T+E) were not significant (p>0.05).

Figure 43. Biogas production rates (ml mℓ⁻¹d⁻¹) as a function of time. Values are averages of four determinations ± standard deviations of biogas produced from the mixture of feedstock and inoculum. For some data points standard deviation bars are smaller than markers.

*Methane content in the biogas*

For the Rocky River study biogas composition was measured during three different days; 6, 17 and 23 (Figure 44). The average methane content (%) at day 6 and 17 of experiment were of 69.2±0.4% and 73.7±1.4 across treatments, respectively (Figure 44). The average methane content at day 23 of study across treatments was of 66.3±1.3%
(Figure 44). Statistical analyses conducted on individual sampling events revealed that differences between group means were not significant (p>0.05).

Figure 44. Methane content in the biogas (%) as a function of time. Values are averages of four determinations ± standard deviations at day 6 and of two for day 17 and 23. For some data points standard deviation bars are smaller than markers.

**Methane yield**

The overall yield achieved for the control (0X-0X) was $296 \pm 23$ mlCH$_4$ gVS$^{-1}$ added (Figure 45). The rates for treatments T1 (0X-0X+T), T2 (0X-0X+T+E), T3 (1X-1X), T4 (1X-1X+T) and T5 (1X-1X+T+E) were of $266 \pm 14$, $258 \pm 7$, $286 \pm 3$, $268 \pm 2$ and $271 \pm 9$ mlCH$_4$ gVS$^{-1}$ added, respectively (Figure 45). Statistical analyses revealed that differences between group means were significant (p<0.05). Tukey-Kramer HSD analysis revealed that the yield measured for the control (0X-0X) was significantly different from those of
T1 (0X-0X+T), T2 (0X-0X+T+E) and T4 (1X-1X+T) but not from T3 (1X-1X) and T5 (1X-1X+T+E).

![Bar chart showing methane yield for different treatments.](image)

Figure 45. Overall methane yield (mlCH\(_4\) gVS\(^{-1}\) added). Values are averages of four determinations ± standard deviations.

**Volatile solids reduction**

The overall volatile solids reduction achieved for the control (0X-0X) and treatments T1 (0X-0X+T), T2 (0X-0X+T+E), T3 (1X-1X), T4 (1X-1X+T) and T5 (1X-1X+T+E) during the 23 day period of study was of 31.0±1.1, 31.3±0.8, 31.6±1.7, 30.7±1.1, 30.3±1.2 and 31.8±2.3%, respectively (Figure 46). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Figure 46. Overall volatile solids reduction (%). Values are averages of four determinations ± standard deviations.

**Final pH**

The final average pH for the control (0X-0X), T1 (0X-0X+T), T2 (0X-0X+T+E), T3 (1X-1X), T4 (1X-1X+T) and T5 (1X-1X+T+E) during the 23 day period of study was of 7.48±0.02, 7.48±0.01, 7.51±0.02, 7.52±0.002, 7.49±0.004 and 7.53±0.01, respectively (Figure 47). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Figure 47. Final pH. Values are averages of two determinations ± standard deviations.

City of Lima batch laboratory-scale studies

Laboratory-scale study 1

Feedstock particle size

Figure 48 shows the SEM of the City of Lima feedstock collected on 1-15-2012 before (a) and after cavitation at 3X-3X (b). The AD study was conducted for a period of 28 days under batch mode. The qualitative analysis revealed that cavitation reduced the particle size of the feedstock to some extent (Figure 48).
Figure 48. Scanning electron microscopy of the City of Lima feedstock collected on 1-15-2012 before (a) and after cavitation at 3X-3X (b).
**Cumulative biogas production**

The total cumulative biogas productions after 28 days of study are shown in Figure 49. The lowest total productions achieved for the control (0X-0X) was of 958±55 ml (Figure 49). This was similar from that measured for T2 (1X-2X) with 956±144 ml (Figure 49). The highest biogas production was observed for T8 (3X-2X) with 1598±114 ml (Figure 49). Productions for the rest of the treatments ranged between 1080 and 1478 ml (Figure 49). Statistical analyses revealed that significant differences (p<0.05) in the total cumulative biogas productions existed between treatment means. Tukey-Kramer HSD analysis revealed that the total cumulative biogas production of the control were significantly different from those measured for T6 (2X-3X), T8 (3X-2X) and T9 (3X-3X). The increases in biogas production between the control and T6 (2X-3X), T8 (3X-2X) and T9 (3X-3X) ranged between 54.3 and 66.8%.
Figure 49. Total cumulative biogas production (ml). Values are averages of three determinations ± standard deviations of biogas produced from the mixture of feedstock and inoculum. Note: The average methane content across treatments was of 67.8±6.0% over the 28 period of study.

**Volatile solids reduction**

The overall volatile solids reduction achieved for the control (0X-0X) over the 28 days period of study was of 27.8±2.0% (Figure 50). The highest reduction was achieved by T8 (3X-2X) with a value of 37.3±6.0% (Figure 50). For the other treatments the values ranged between 27.5 and 36.9% (Figure 50). Statistical analysis revealed that significant differences in the volatile solids reduction extent existed between treatment means (p<0.05). Tukey-Kramer HSD analysis revealed that differences were significant between the control (0X-0X) and T9 (3X-3X). The increase in volatile solids reduction of T9 (3X-3X) over the control was of 34.2%.
Laboratory-scale study 2

*Feedstock particle size distribution*

Figure 51 shows the particle size analysis of the City of Lima feedstock collected on 9-22-2013. The AD study was conducted for a period of 20 days under batch mode.

The uncavitated (0X-0X) feedstock distribution was characterized by one major peak with centered apex located at 77 µm (Figure 51). The cavitated T1 (1X-2X) and T2 (2X-4X) feedstocks showed one major peak with apex centered at 22 and 17 µm (Figure 51).
Figure 51. Particle size analysis of City of Lima feedstock collected on 9-22-2013.

**Initial total and soluble chemical oxygen demand**

The total initial COD of the control (0X-0X), T1 (1X-2X) and T2 (2X-4X) was of 44133±9127, 42000±4276 and 52133±5460 mgL⁻¹, respectively (Figure 52a). Statistical analysis revealed that differences between group means were not significant (\(p>0.05\)).

The soluble COD of the control (0X-0X), T1 (1X-2X) and T2 (2X-4X) was of 762±9, 929±9 and 915±13 mgL⁻¹, respectively (Figure 52b). Statistical analysis revealed that significant differences existed between group means (\(p<0.05\)). Tukey-Kramer HSD analysis revealed that the soluble COD of the control (0X-0X) was significantly different from T1 (1X-2X) and T2 (2X-4X). Moreover, differences between T1 (1X-2X) and T2 (2X-4X) were not significant. The increase observed in soluble COD for the cavitated samples was of 21.9±4 and 20.0±3% when compared with T1 (1X-2X) and T2 (2X-4X), respectively.
Figure 52. Total (a) and soluble (b) chemical oxygen demand of City of Lima uncavitated (T1) and cavitated (T2, T3) feedstocks (mg L$^{-1}$). Results are averages of three determinations.

**Cumulative biogas production**

Cumulative biogas productions are shown in Figure 53. Cumulative biogas productions over a 1 day period reached 326±12, 347±13 and 334±18 ml for the control (0X-0X), T1 (1X-2X) and T2 (2X-4X), respectively (Figure 53). Statistical analysis revealed that differences between group means at day 1 were not significant (p>0.05).
Day 2 cumulative biogas productions reached 434±26, 478±23 and 450±20 ml for the control (0X-0X), T1 (1X-2X) and T2 (2X-4X), respectively (Figure 53). Statistical analysis revealed that differences between group means at day 2 were significant (p<0.05). Tukey-Kramer HSD analysis revealed that the cumulative biogas production of T1 (1X-2X) was significantly different from the control and T2 (2X-4X). The increase of T1 (1X-2X) over the control was of 10.1%.

Cumulative biogas productions over the 20 day experimental period reached 1007±11, 1036±18 and 1001±16 ml for the control (0X-0X), T1 (1X-2X) and T2 (2X-4X), respectively (Figure 53). Statistical analysis revealed that differences between final group means were not significant (p>0.05).

![Figure 53. Cumulative biogas production (ml) as a function of time. Values are averages of five determinations ± standard deviations of biogas produced from the mixture of feedstock and inoculum. For some data points standard deviation bars are smaller than markers.](image-url)
Biogas production rates

Biogas productions rates are shown in Figure 54. Biogas productions rates in the first day of study were of 0.65±0.03, 0.69±0.03 and 0.70±0.04 ml ml⁻¹ d⁻¹ for the control (0X-0X), T1 (1X-2X) and T2 (2X-4X), respectively (Figure 54). Statistical analysis revealed that differences between group means at day 1 were not significant (p>0.05).

Day 2 biogas production rates reached 0.21±0.03, 0.26±0.02 and 0.23±0.01 ml ml⁻¹ d⁻¹ for the control (0X-0X), T1 (1X-2X) and T2 (2X-4X), respectively (Figure 54). Statistical analysis revealed that differences between group means at day 2 were significant (p<0.05). Tukey-Kramer HSD analysis revealed that the biogas production rate of T1 (1X-2X) was significantly different from the control and T2 (2X-4X). This represents a 22% increase in the biogas production rates of T1 (1X-2X) over the control.

Biogas productions rates decreased in all treatments until reaching values that ranged between 0.01 and 0.02 ml ml⁻¹ d⁻¹ during day 20 of study (Figure 54). Statistical analysis revealed that differences in biogas production rates between group means between days 2 and 20 were not significant (p>0.05).
Figure 54. Biogas production rates (ml ml\(^{-1}\)d\(^{-1}\)) as a function of time. Values are averages of five determinations ± standard deviations of biogas produced from the mixture of feedstock and inoculum. For some data points standard deviation bars are smaller than markers.

**Methane content in the biogas**

Initial methane content in the biogas ranged between 63.9 and 66.4% across treatments (Figure 55). Methane content in the biogas then remained virtually constant among treatments during the 20 day period of study with values ranging between 63.2 and 66.3% (Figure 55). Statistical analysis revealed that differences between group means over the 20 day period of study were not significant (p>0.05).
Figure 55. Methane content in the biogas (%) as a function of time. Values are averages of five determinations ± standard deviations. For some data points standard deviation bars are smaller than markers.

**Methane yield**

The overall methane yield achieved for the control (0X-0X), T1 (1X-2X) and T2 (2X-4X) over the 20 day period of study was of 111±1, 114±2 and 110±2 mlCH$_4$ gVS$^{-1}$ added, respectively (Figure 56). Statistical analysis revealed that differences between group means were significant (p<0.05). Further analysis revealed that T1 (1X-2X) was significantly different when compared to the two other treatments. The increase in methane yield observed for T1 (1X-2X) over the control was of 3.0%.
Volatile solids reduction

The overall volatile solids reduction achieved for the control (0X-0X), T1 (1X-2X) and T2 (2X-4X) during the 20 day period of study was of 17.0±3.1, 18.4±2.6 and 18.2±1.2%, respectively (Figure 57). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Figure 57. Overall volatile solids reduction (%). Values are averages of five determinations ± standard deviations.

**Final pH**

The final average pH for the control (0X-0X), T1 (1X-2X) and T2 (2X-4X) during the 20 day period of study was of 7.75±0.05, 7.61±0.04 and 7.68±0.06, respectively (Figure 58). Statistical analyses revealed that differences between group means were not significant (p>0.05).
Discussion

Laboratory and long-term pilot-scale experiments were conducted to study the effects of cavitation pretreatment on particle size reduction and reactor performance in mesophilic anaerobic digesters treating mixed primary and secondary sludges. A complete description of the experimental treatments can be found in Table 10, Table 11, Table 12 and Table 13. The pilot-scale study was operated on a semi-continuous mode over a 50 day period. Five different laboratory-scale experiments were conducted in batch mode for 20 to 30 days with feedstocks obtained from different wastewater treatment plants (Table 15).

City of Wooster feedstocks were collected from the same location during four different dates in order to determine the variability in particle size distributions of
uncavitated substrates in wastewater treatment plants (Table 15). The particle size analysis conducted on the uncavitated (0X-0X) substrates was characterized by two major peaks located at approximately 100 and 200 µm for feedstocks 1, 2 and 3 whereas only one peak located at 67 µm for feedstock 4 (Figure 16). Moreover, feedstock four had the highest content of secondary sludge on the mixture and the lowest volatiles solids content.

City of Wooster feedstocks 1 and 2 were processed using different cavitation process conditions (Figure 16). The average total solids content of feedstocks 1 and 2 ranged between 2.16 and 2.34% (Table 15). Particle size analyses conducted on feedstock 1 showed that when the substrate was processed at fewer passes (1X) the particle size distribution was characterized by one major peak ranging between 22 and 26 µm (Figure 16a). When the same feedstock was processed with more passes (2X), one major peak was also observed with centered apex at 19 µm (Figure 16a). For City of Wooster feedstock 2 results showed that at the lowest pressure (1X) the distribution was characterized by one major peak located at 34 µm (Figure 16b). When the pressure drop was increased to 1.5X and 2X the distributions were characterized also by one major peak located at 22 µm in both conditions (Figure 16b). The results of these two analysis showed that a substantial reduction in particle size could be achieved when the feedstocks are passed 1X times through the cavitation unit at 1.5 and 2X pressure drops.

The effect of sludge characteristics on the extent of particle size reduction was evaluated by comparing the four City of Wooster feedstocks cavitated using the same process conditions (1X-2X). Particle size analyses showed that after cavitation pretreatment, the distributions were characterized by one major peak for all feedstocks
The peaks were centered at 26, 22, 39 and 20 µm for feedstocks 1, 2, and 4, respectively (Figure 16). Results of this study showed that similar reductions on particle size were achieved for feedstocks having similar total solids content (2.16 to 2.86%). The lowest particle size reduction was achieved for the feedstock that had the highest percent of initial total solids, feedstock 3 (TS=3.55±0.02%).

In a separate study we conducted cavitation pretreatment on non-thickened secondary sludges (Appendix B) obtained from The City of Wooster wastewater treatment secondary sedimentation tank. Results showed that substantial reductions were achieved at the total solids content as low as 0.38±0.02% (Appendix B). The uncavitated (0X-0X) and cavitated T1 (1X-2X) feedstocks particle size distribution analyses were characterized by one major peak with apex centered at 59 and 20 µm, respectively (Appendix B). The results of this study suggest that the cavitation technology used during our study is also effective at low total solids such as those found right after aeration tanks. This could improve the dewaterability of secondary sludge during thickening and centrifuging in wastewater treatment operations.

A different study conducted with Rocky River primary sludge feedstock did not show substantial particle size reductions after substrates were cavitated at the lowest process conditions (1X-1X). This inability of cavitation to influence particle size could be related to the total solids content of the feedstocks (TS=5.21±0.04%). These results are different from those observed for the City of Wooster feedstock 2 (Figure 16b) which showed a substantial reduction on particle size could be achieved when a feedstock containing 2.16±0.02% total solids was processed at the same cavitation conditions (1X-1X).
Particle size reduction analyses conducted on the feedstocks obtained from the City of Lima with higher content of secondary sludge (2.78±0.02% TS) showed substantial reductions at 1X-2X (Figure 51). Reductions achieved by increasing the cavitation process conditions from 1X-2X to 2X-4X were small (Figure 51). SEM analysis conducted in a feedstock obtained from the same location in a different date also showed particle size reductions when cavitation process conditions were as high as 3X and 3X passes and pressures, respectively Figure 48.

The effectiveness of ultrasonic pretreatment on sludge disintegration is influenced by many factors including process conditions and substrate chemical and physical properties such as composition, initial particle size and total solids content (Show et al., 2007). The results of previous studies on the relationship between total solids content and cavitation effectiveness are not consistent. Some authors theorize that at higher solids content, cavitation bubbles should have higher probabilities of impacting sludge particles resulting in higher disintegration rates (Carrère et al., 2010). On the other hand, studies conducted by Khanal et al. (2007) report that increases in the total solids content lead to adverse effects on particle disintegration. According to Show et al. (2007), increases in the total solids content increase fluid viscosity that hinders cavitation bubbles formation. Other studies conducted on secondary sludge have reported significant disintegration rates with substrates containing total solids ranging between 2.3 and 3.2% (Show et al., 2007).

Studies conducted by Rivard & Nagle (1996) on ultrasound pretreatment of mixed sludges found that a decrease in the disintegration extent was observed when the total solids content increased above 1%. Show et al. (2007) reported significant particle
disruption at total solids content below 2.9% and marginal affects at 3.8%. However, studies conducted by Eskicioglu et al. (2008) on mixed sludges reported significant particle size reductions with feedstock total solids content as high as 4.1%. Our studies on mixed sludges showed that lower particle size reduction extents were achieved for the feedstock containing total solids contents of 5.2%. Moreover, the extent of particle reduction observed in our studies at 5.2% total solids could also be related to the low cavitation pressure drop used during feedstock pretreatment. This may not have been sufficient to achieve cavitation conditions. Our studies showed that substantial reductions in particle size were achieved when the solids content of mixed sludges ranged between 2.16 and 2.86%.

A different study was conducted to track the changes in particle size distribution inside pilot-digesters treating uncavitated and cavitated City of Wooster feedstocks (Figure 18) 1, 2, 3 and 4 (Table 10). Particle size analyses conducted on all four initial uncavitated feedstocks were characterized by two major peaks located at 100 and 200 µm for feeds 1, 2 and 3 and only one major peak for feed 4 located at 67 µm (Figure 16). Results of the digester operated with uncavitated feedstock showed that the anaerobic digestion process alone reduced the particle size to 59 µm after 7 days (Figure 18a). Then, the particle size remained stable as observed in our 50 day study. When cavitated feedstocks were used, a lower reduction was observed after 7 days of study with one major peak located at 45 µm (Figure 18b). This peak was located at 30 µm during the final week of study (Figure 18b) when the fraction of cavitated material inside the reactor was approximately 87% (Figure 17). These results indicate that biological processes
inside anaerobic digesters both result in effluent with smaller particle sizes but that cavitation treatment leads to smaller particle sizes.

Ultrasound applications for substrates containing mostly primary sludge are particularly important for dewatering processes in wastewater treatment systems (Carrère et al., 2010; Kargar & Mahvi, 2012). The ultrasound pretreatment has the ability to induce physicochemical changes on sludge characteristics including structure and degree of hydration resulting in increased settling and dewatering rates (Zhang et al., 2006). Higher dewaterability rates can increase the effectiveness of thickeners and centrifuges used in daily operations to separate the solids from the water fractions reducing the costs associated with sludge processing, transportation and disposal and improving the overall cost-effectiveness of wastewater treatment operations (Novak, 2006). In addition, other costs such as those associated with polymer addition for improving sludge dewatering could also be considered (Crawford, 1990).

Another advantage of the technology used during our study to reduce the particle size of different feedstocks is that the cavitation process is conducted under continuous flow conditions. This is different from other forms of pretreatment were long residence times are needed to achieve substantial particle size reductions. Studies conducted using ultrasonic sonication on primary and secondary sludges by Mao et al. (2004) reported substantial particle size reductions within five minutes of pretreatment for both feedstocks. For studies conducted with secondary sludges pretreatments times can be found from 6-54 seconds (Pérez-Elvira et al., 2009), 20 to 30 minutes (Chu et al., 2002; Onyeche et al., 2002a) and as high a 150 min (Tiehm et al., 2001).
Stability parameters were measured for the pilot digesters along the 50 day period of study and included pH, alkalinity and volatile fatty acids content inside the digesters. The pH remained constant over the 50 day period of study with values ranging between 7.50 and 7.65 between pilot digesters (Figure 26). Optimum pH concentrations for anaerobic digestion range between 6.6 and 7.6 (Ten Brummeler et al., 1985). However, changes in pH values are usually accompanied by increases in total volatile fatty acid in systems with low alkalinity-buffering capacity (Griffin et al., 1998). Moreover, the concentration of fatty acids in the pilot digesters ranged between 40-100 mg Kg\(^{-1}\) and substantial accumulations were not observed during the entire duration of the study (Figure 27). In addition, the overall average alkalinity concentration inside the pilot digesters treating uncavitated and cavitated feedstocks were of 6211±36 and 6094±54 mgCaCO\(_3\)eq kg\(^{-1}\) (Figure 28) and it was sufficient to avoid a pH decline. Overall, these results indicate that there were no adverse effects of feedstock cavitation pretreatment on reactor stability for the conditions evaluated during this study.

Daily biogas production analyses of the pilot-scale study showed that the highest values were measured during the first four days of study (Figure 19). This was reflected in the average weekly biogas production (Figure 20) and biogas production rates (Figure 21) for both the control and treatment digesters. During the second week of study a decrease in biogas production was observed for both digesters (Figure 19). Decreases in the average weekly biogas productions (Figure 19) and biogas production rates continued until the third week of study (Figure 21). However, the differences observed for the first three weeks of study were not substantial when compared between digesters and between weeks.
The decreases in biogas production observed in the pilot digesters after the first week of study could be related to the total and volatile solids content of the inoculum and feedstocks 1 and 2. Before the experiment was started, both digesters were inoculated with 25 gallons of inoculum containing 3.09±0.02 and 69.0±1.0% of total and volatiles solids, respectively (Table 14). Both reactors were then given three days without feeding to allow microorganisms to consume readily available substrates. Digestors were then fed with feedstocks (Table 15) containing lower total solids content (2.16 to 2.34%). While the reactors were operated with feedstocks 1 and 2 the organic loading rates were of 0.89 and 0.77 kgVS m$^{-3}$ d$^{-1}$, respectively.

During the fourth week of study the average weekly biogas productions increased for both pilot digestors (Figure 20). This increase was sustained until week 6 of study. The stability in biogas production observed during weeks 4, 5 and 6 could be related to the solids content of feedstock 3. Feedstock 3 was fed to both digesters according to the experiment treatments and the total and volatile solids content were of 3.55±0.02 and 62.1±0.6%, respectively. The organic loading rate applied to the digestors while operating with feedstock 3 was the highest used during the 50 day period study (1.06 kgVS m$^{-3}$ d$^{-1}$). Moreover, differences in biogas productions were not substantial between digesters and between weeks. For the last week of study, biogas production decreases were observed for both digesters. During this period the digestors were operated with 0.78 kgVS m$^{-3}$ d$^{-1}$ according to the experimental treatments. The feedstock used during the last week had the highest content of secondary sludge and the lowest volatile solids content when compared with the other four feedstocks obtained from the same location.
Overall, no substantial differences were observed for biogas production between pilot digesters operated with uncavitated or cavitated feedstocks. However, from the particle size analyses it was demonstrated that cavitation technology substantially reduced the feedstock particle size. It was then hypothesized that the digester operated with pretreated feedstock could be producing most of the daily amount of biogas during the first few hours of the day. For this, biogas production was measured on an hourly basis two different days a week during weeks 3, 5 and 7 of study (Figure 22). This hypothesis was disproven since both reactors produced biogas at a nearly constant rate over the 24 hour period (Figure 22).

The effects of feedstock pretreatment on methane content in the biogas was measured on a weekly basis for both pilot-scale digesters (Figure 23). Results showed that the overall methane content in the biogas produced from the pilot digesters ranged between 64 and 68% across treatments (Figure 23). The values for methane content observed during this study are within the range of those reported for stable digesters, 60-70% (Appels et al., 2008).

Methane content, biogas production and initial volatile solids added to the pilot-digesters were used to calculate the average weekly methane yield for each digester (Figure 24). The methane yield is widely used to evaluate the efficiency of the system in important transient phases. Methane yields calculated for both digesters were similar along the entire period study (Figure 24). Some week to week variability was observed at weeks 1 and 7 of study. During week 7, a decrease in the methane yield was observed for both digesters (Figure 24). Moreover, differences in methane yield did not appear to be significant between weeks of study (Figure 24).
Overall the yield values ranged between 150 and 190 LCH₄ kgVS⁻¹ added (Figure 24). Our pilot studies did not show any substantial differences on methane yields between the reactors operated with uncavitated or cavitated sludge. Other studies conducted using primary sludge have reported methane yields of 159 and 240 on continuous feeding systems (Kabouris et al., 2009) and of 470 LCH₄ kgVS⁻¹ added on batch experiments (Kabouris et al., 2007). Moreover, variability in methane yields of sewage sludge could be influenced by many factors including substrate characteristics (Nizami et al., 2009) and anaerobic digestion process parameters, among others.

Results obtained during the 50 day pilot-study revealed that differences in volatile solids reduction between the two digesters were not substantial (Figure 25). The average volatile solids reduction for the reactor operated with uncavitated feedstock ranged between 43.0 and 52.8% (Figure 25). The reactor operated with cavitated feedstocks achieved reductions that ranged between 37.0 and 55.6% (Figure 25).

Two separate batch laboratory-scale studies were conducted on City of Wooster feedstocks 1 and 3 collected on 7-18-2013 (laboratory-scale 1) and 8-16-2013 (laboratory-scale 2), respectively (Table 15). Laboratory-scale 1 evaluated the effects of cavitation pretreatment at 5 different passes and pressures combinations (Table 11). Laboratory-scale 2 evaluated the effects of cavitation with the City of Wooster feedstock containing the highest solids content at 2 different process combinations (Table 11). Results obtained from the laboratory-scale 1 and 2 studies revealed that there were no significant differences between the reactors loaded with uncavitated or cavitated feedstocks on performance parameters. Overall, even though cavitation pretreatment substantially reduced the particle size of mixed sludges, this did not improve or affect the
overall performance of the anaerobic digesters under the conditions used for these studies.

A different batch laboratory-scale study was conducted with City of Rocky River mixed sludge containing mostly primary sludge (Table 15). Performance results showed that pretreated feedstocks (i.e. either thermal; enzymatic, cavitation, and combinations of these three) achieved higher biogas production rates during the first 4 days of study when compared to the reactors loaded with uncavitated (Figure 43). Moreover, differences between pretreatments methods were not significant. An 12.3±5% increase was observed for the cavitated feedstocks when compared to the untreated sludge (Figure 43). After day 4 of study, higher biogas productions were measured for the reactor loaded with uncavitated feedstock. Overall, final cumulative biogas productions were not significantly different between the reactors loaded with uncavitated or pretreated feedstocks. This conducted was reported by Eskicioglu et al. (2008) in which ultrasound of primary sludges showed an increase in initial biogas production rates but not in the final cumulative values.

The City of Lima laboratory-scale study 1 showed significant increases in the total biogas production and volatile solids reduction extent. Increases in biogas ranged between 54.3 and 66.8% when feedstocks containing mostly secondary sludge were cavitated at 2X-3X, 3X-2X and 3X-3X (Figure 49). Moreover, differences in productions between pretreatments at 2X-3X, 3X-2X and 3X-3X were not significant. An 34.2% increase in volatile solids reduction over the control was observed (Figure 50) when the feedstock was cavitated with the highest processing conditions (3X-3X). Increases in biogas productions up to 58% have also been reported when for studies conducted in
different scales and different ultrasound pretreatment technologies on (Table 16). Increases due to ultrasound pretreatment on volatile solids reduction ranging between 17 and 57% have also been reported in feedstocks containing mostly secondary sludge (Table 16).

The City of Lima laboratory-scale study 2 showed increases in soluble COD over that of the control with values ranging between 20.0 and 21.9% when feedstocks containing about 80% of secondary sludge (v/v) were cavitated at 1X-2X and 2X-4X process conditions (Figure 52a). In addition, differences in soluble COD increases between cavitation conditions 1X-2X and 2X-4X were not significant (Figure 52b). Other studies conducted to evaluate the effects of ultrasound pretreatment on soluble COD content in secondary sludges have reported increases of 35% for the same parameter (Table 16).

Increases of 11±2.1% in biogas production over those measured for the control were observed for the digesters loaded with cavitated feedstock (1X-2X) during the second day of study only (Figure 53). This was reflected in the biogas production rates when comparing the same treatments during the same day with increases of 22±3.4% (Figure 54). However, differences in the final cumulative biogas productions were not significant between the controls and the digesters operated with cavitated feedstocks, as well as between the two different cavitation process conditions (Figure 53). In addition, no increases in volatile solids reductions were observed for the digesters operated with cavitated feedstocks and the control (Figure 57). Moreover, when cavitation was applied at 1X-2X the overall methane yield increased by 3.0±1.2% when compared to that of the control by (Figure 56).
Table 16. Performance data of different anaerobic processes applied to secondary sludges.

<table>
<thead>
<tr>
<th>Scale</th>
<th>Substrate*</th>
<th>Pretreatment condition</th>
<th>Feeding mode</th>
<th>Temperature</th>
<th>Resultsa,b</th>
<th>Referencesc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab</td>
<td>Secondary</td>
<td>Ultrasound reactor, 41 kHz, 150 min.</td>
<td>Semi-</td>
<td>37°C</td>
<td>Increases up to 57 and 42% on VS reduction and biogas production, respectively.</td>
<td>A</td>
</tr>
<tr>
<td>Lab</td>
<td>Secondary</td>
<td>Ultrasonic homogenizer, 41 kHz, 225 W.</td>
<td>Batch</td>
<td>35-37°C</td>
<td>Increases of 35 and 32% on initial soluble COD and cumulative biogas production, respectively.</td>
<td>B</td>
</tr>
<tr>
<td>Lab</td>
<td>Secondary</td>
<td>Ultrasound processor, 24 kHz, 255 W, 2-4 min.</td>
<td>Semi-</td>
<td>37°C</td>
<td>Increases of 17 and 36% on VS reduction and biogas production, respectively.</td>
<td>C</td>
</tr>
<tr>
<td>Pilot</td>
<td>Secondary</td>
<td>Continuous ultrasound homogenizer, 30 kHz m$^3$, 100 W.</td>
<td>Continuous</td>
<td>35-37°C</td>
<td>Increases of 25 and 37% on VS reduction and biogas production, respectively.</td>
<td>D</td>
</tr>
<tr>
<td>Full-scale</td>
<td>1/3 primary (TS:1 to 2%) + 2/3 Secondary (TS:2-4%)</td>
<td>Five sets of 20 kHz ultrasound generators operated at 3 kW.</td>
<td>Continuous</td>
<td>Not specified</td>
<td>Increases between 18 and 58% in biogas production.</td>
<td>E</td>
</tr>
</tbody>
</table>

*a WAS: Waste activated sludge, COD: chemical oxygen demand. b Compared to the AD of untreated feedstock. b Biogas contains a mixture of predominantly methane and carbon dioxide. c References: A = (Tiehm et al., 2001), B = (Bougrier et al., 2005), C = (Braguglia et al., 2008), D = (Pérez-Elvira et al., 2009), E = (Xie et al., 2007).
Overall, particle size analysis conducted in the City of Wooster feedstocks containing 70-80% (v/v) primary sludge obtained in four different dates revealed that substantial reductions were achieved for feeds containing total solids ranging between 2.16 and 3.55% cavitated at process conditions ranging between 1X-1X and 2X-3X using the same bench-scale cavitation unit. Pilot-scale anaerobic digestion studies conducted with City of Wooster feedstocks during a 50 day period showed that cavitation pretreatment did not affect or improve reactor performance under the conditions evaluated during this study. In addition, parameters used to measure AD process instability such as organic acids accumulation and alkalinity concentrations remained within ranges to avoid a pH drop below levels reported to cause process failure. Furthermore, batch-laboratory scale studies did not show improvements in reactor performance for the digesters loaded with cavitated feedstocks as well.

A separate AD study was conducted with feedstock obtained from the City of Ricky River containing mostly primary sludge. Particle size analysis revealed that cavitation pretreatment at process conditions 1X-1X was not enough to cause substantial particle size reductions in a feedstock containing 5.21±0.04% solids. Cavitation pretreatment was performed using a pilot-scale unit. Moreover, the marginal reductions in particle size could have influenced the 12.3±5% increase in biogas production observed in the first 3 days of study. However, this increase did not affect the final cumulative biogas production as no significant differences were observed when the treatments were compared to that of the control. Results from this study confirm that pretreatment on feedstocks containing mostly primary sludge could have the potential to increase biogas production during initial days of the anaerobic digestion process which
could be reflected in shorter retention times and higher organic loading rates. However, more studies are needed to evaluate the effects of cavitation pretreatment and increased organic loading rates on anaerobic digesters treating mixed sludges with higher content of the primary portion.

Studies conducted with City of Lima feedstocks containing mostly secondary sludge showed that substantial reductions in particle size could be achieved when cavitation process conditions were as low as 1X-2X and total solids content of 2.78±0.02%. A first study conducted with City of Lima feed 1 containing 52.6±1.0% volatile solids showed improvements in biogas productions up to 66.8±8% for the batch laboratory-scale reactors operated with cavitated substrates when compared to values measured for the controls. For the same study, an 34.2±4% increase in volatile solids reduction of T9 (3X-3X) over the control was observed. Moreover, no significant increases in the ultimate biogas productions were observed when a second study was conducted with feedstock obtained from the same location. Moreover, the chemical properties analysis conducted in the second feedstock revealed that the content of volatile solids was higher (VS= 61.5±0.6%) than that of the feedstock used in the first study. The volatile solids content in the feedstock used for the second study might be an indication that mixture of sludge contained a higher fraction of primary substrate. Results of performance could also have been influenced by the cavitation unit used for feedstock pretreatment as different units were used to process feedstocks for each study. However, more studies in this area are needed to elucidate the effects of cavitation unit design including pipe diameter, shear forces and other factors such as feedstock characteristics on performance and stability of anaerobic digesters.
Conclusion

In this study, anaerobic digestion experiments were conducted to study the effects of feedstock pretreatment using a novel hydrodynamic cavitation technology on particle size reduction and reactor performance of mesophilic anaerobic digesters treating mixed sewage sludge obtained from three different wastewater treatment plants in Ohio.

Studies conducted in feedstocks containing mostly primary sludge with total solids content ranging between 2.16 and 3.55% showed that hydrodynamic cavitation was able to achieve substantial reductions in substrate particle size. Further analysis revealed that low pressure drop (1X) and less number of passes (1X) were sufficient to achieve alterations in the overall particle size distribution. Moreover, reductions in particle size were marginal when a feedstock containing 5.21% total solids was processed at the lowest cavitation process conditions (1X-1X).

Pretreatment in feedstocks containing mostly secondary sludge also showed substantial reductions in particle size analysis with total solids content ranging between 0.38 and 2.78% with cavitation processing conditions ranging between 1X-2X and 3X-3X.

The results of the semi-continuous pilot-scale anaerobic digestion studies conducted over a 50 day period using mixed sludge with higher content of the primary portion revealed even though substantial particle size reductions were measured, this did not affect or improve the stability nor the performance of the reactors operated with cavitated feedstock.

Laboratory-scale studies conducted in batch mode with feedstocks containing mostly primary sludge showed that cavitation pretreatment increased biogas production.
rates by 12.3±5% during the first three days of study. Moreover, differences in the final cumulative biogas productions were not significant.

Cavitation pretreatment of mixed sludge feedstocks containing 80% secondary and 20% primary increased soluble COD by 21.9±4%.

Laboratory-scale batch anaerobic digestion studies conducted with feedstocks containing mostly secondary sludge showed that cavitation pretreatment increased total biogas productions and volatile solids reduction up to 66.8±8 and 34.2±4%, respectively.

Results from these preliminary studies suggest that hydrodynamic cavitation is a simple method with the potential to achieve substantial particle size reductions in different sewage sludge substrates and has the potential to improve not only wastewater treatment operations, but also anaerobic digestion processes.

**Acknowledgements**

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Chapter 5 – Conclusion and recommendations

Conclusions

In this study, the relative biodegradability of polymeric materials used for various commercial applications was investigated under composting, soil incubation and AD conditions. Another area of study included the evaluation of the ability of hydrodynamic cavitation for pretreatment of municipal sewage sludge for particle size reduction and effects in reactor performance.

The first study demonstrated that although certain bio-based plastics and natural fibers biodegrade to an appreciable extent in the three environments, only a polyhydroxyalkanoate-based resin biodegraded to significant extents during the time scale of composting and anaerobic digestion processes used for solid waste management. Moreover, no significant degradation was observed for polyethylene or polypropylene plastics or the same plastics amended with commercial additives meant to confer biodegradability.

The second study showed that PUs made from 100% bio-based polyols biodegraded different to the petroleum-based analogs in soil incubation. Structural analyses revealed that both urethane and ester-related segments were susceptible to microbial attack in samples containing at least 50% of petroleum-based polyols. Moreover, ester groups in the polyol side of the polymer were the preferred site of attack in PUs made from 100% bio-based polyols.
The third study revealed that hydrodynamic cavitation was able to achieve substantial reductions in substrate particle size in mixed sludge feedstocks containing either mostly primary or secondary sludge with total solids ranging between 0.38 and 3.55%. Biomethanation studies showed that cavitation did not improve or affect the extent of biodegradation in mesophilic digesters treating primary sludge. Furthermore, increases of 66.8 and 34.2% were observed in total biogas production and volatile solids reductions when digesters were loaded with feedstocks containing mostly secondary sludge, respectively.

**Future studies**

- The relative biodegradability of novel plastics should be studied under all possible scenarios of degradation to understand the interactions of these materials with the environment. Other studies should include a combination of different forms of degradation including photodegradation, chemical, mechanical and other forms that best mimic the different fates of these materials in natural and waste management scenarios.
- Studies conducted in degradation of novel polymers should include not only the extent of conversion under different conditions, but the analysis of structural and chemical changes during transient phases of the process.
- Our studies showed that some degradation takes place in polyurethane foams produced from bio-based feedstocks. More studies on polymer design need to be conducted to augment or decrease degradation due to microbial attack to satisfy the needs of different commercial applications.
- Chemical changes in the structure of the polyurethane foams were only analyzed after a 50-day composting period. Moreover, structural analyses should also be conducted under soil and anaerobic conditions to understand the microbial degradation mechanisms used in different scenarios.

- Preliminary studies conducted on hydrodynamic cavitation demonstrated that substantial reductions in particle size were achieved in feedstocks containing mixed municipal sewage sludge. More studies on the effects of not only substrate total solids but other sewage sludge components such as fats content on cavitation efficiency need to be conducted. This will aid the design of cavitation units for specific applications in the overall wastewater treatment and anaerobic digestion processes.

- More studies on cavitation pretreatment of sewage sludge need to be conducted to understand the effects on dewatering and settling rates as reducing volumes associated with wastewater treatments is still a major challenge.

- A Net Energy Value study needs to be conducted to understand the energy requirements of hydrodynamic cavitation technology as an aid for wastewater treatment, and balances with the improvements in the energy output of the anaerobic digestion system.
References


ASCE. 2000. *Conveyance of residuals from water and wastewater treatment*. American Society of Civil Engineers, Reston, VA.


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Appendix A - Schematic diagram of the composting system used for the polymer biodegradability studies.
Appendix B - Particle size analysis of City of Wooster unthickened secondary sludge.

![Graph showing particle size distribution](image-url)